Soluble T-cadherin promotes pancreatic β-cell proliferation by upregulating Notch signaling
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SUMMARY
Endogenous humoral factors that link systemic and/or local insulin demand to pancreatic β-cells have not been identified. Here, we demonstrated that T-cadherin, a unique glycosylphosphatidylinositol-anchored cadherin primarily expressed in vascular endothelial cells and cardiac and skeletal muscle cells, but not in pancreatic β-cells, was secreted as soluble forms and was important for β-cell proliferation. Cdh13 (T-cadherin) knockout mice exhibited impaired glucose handling due to attenuated β-cell proliferation under high-fat diet conditions. The gene expression analyses indicated the impairment in cell cycle and Notch signaling in the islets of T-cadherin knockout mice under high-fat diet conditions. In streptozotocin-induced diabetes, the replacement of soluble T-cadherin improved β-cell mass and blood glucose levels in T-cadherin knockout mice. Recombinant soluble T-cadherin upregulated Notch signaling in cultured murine islets. We concluded that soluble T-cadherin could work as an endogenous humoral factor whose signaling pathways including Notch signaling regulate β-cell proliferation under diabetic conditions in mice.

INTRODUCTION
The failure of adaptive pancreatic β-cell proliferation and resultant insulin insufficiency in response to increased insulin demand is the key feature of type 2 diabetes. To overcome this, several important mechanisms governing β-cell proliferation have been investigated (Bartolome et al., 2019; De Groef et al., 2016; El Ouamami et al., 2016; Eom et al., 2021; Fajas et al., 2004; Jimenez-Gonzalez et al., 2013; Kim et al., 2017; Ma et al., 2021; Oger et al., 2020; Rosado-Olivieri et al., 2020). Pancreatic β-cells can adaptively proliferate in response to not only insulin resistance but also β-cell deficiencies (Yin et al., 2006). Such adaptive proliferation is the primary mechanism of β-cell expansion, which works even in adult humans (Dor et al., 2004; Meier et al., 2008; Teta et al., 2007). Recent progress in exploiting single-cell RNA sequencing techniques has revealed the presence of proliferative and nonproliferative β-cells (Rosado-Olivieri et al., 2020; Tatsuoka et al., 2020). However, the humoral factors that physiologically regulate β-cell proliferation in response to insulin demands are not yet fully understood.

T-cadherin is a member of the classical cadherins but is unique in terms of having a glycosylphosphatidylinositol (GPI) anchor on its C-terminus (Hulpiau and Van Roy, 2009). A proportion of T-cadherin expressed on the cell surface has an unprocessed prodomain on its N-terminus (Ranscht and Dours-Zimmermann, 1991). T-cadherin is expressed in the heart, muscle, vascular endothelial cells, and mesenchymal stem/stromal cells (Denzel et al., 2010; Nakamura et al., 2020) and serves as a specific receptor for adiponectin, an adipose tissue-specific secretory factor (Fukuda et al., 2017; Kita et al., 2019a). In our previous studies, we demonstrated that adiponectin binds to T-cadherin and enhances the biogenesis and secretion of exosomes and small extracellular vesicles (EVs) in T-cadherin-expressing cells (Kita et al., 2019b; Nakamura et al., 2020; Obata et al., 2018; Tanaka et al., 2019; Tsugawa-Shimizu et al., 2021).

Recently, we developed new monoclonal antibodies against T-cadherin and identified three novel forms of soluble T-cadherin in addition to the previously identified EV-associated T-cadherin in human plasma: an approximately 130-kDa full-length form, an approximately 100-kDa form without the prodomain, and a...
Figure 1. T-cadherin deficiency in mice impairs glucose tolerance under HFD conditions due to defects in the adaptive increase in pancreatic β-cell mass

(A) Protocol of NC or HFD feeding. Eight-week-old male mice were fed either NC or an HFD for 6 weeks.

(B) Body weight transition (**p < 0.01; unpaired t test CT_HFD versus KO_HFD, n = 12 CT_NC, n = 10 KO_NC, n = 27 CT_HFD, n = 33 KO_HFD).

(C) Oral glucose tolerance test. After overnight fasting, mice were orally administered 2 g/kg body weight glucose (*p < 0.05, **p < 0.01, ***p < 0.001; Blood glucose: unpaired t test CT_HFD versus KO_HFD, Area under curve (AUC): unpaired t test. n = 12 CT_NC, n = 10 KO_NC, n = 27 CT_HFD, n = 33 KO_HFD).

(D) Plasma insulin in the oral glucose tolerance test (*p < 0.05, ***p < 0.001; unpaired t test. n = 27 CT_HFD, n = 33 KO_HFD).

(E) Insulin tolerance test. After 4 h of fasting, mice were intraperitoneally administered 0.75 units/kg body weight human insulin (unpaired t test, n = 15 CT_HFD, n = 21 KO_HFD).

(F) Representative images of pancreases from CT and KO mice fed an HFD with DAB staining (the upper row) and immunohistochemical staining for insulin, glucagon, and DAPI (the lower row). Scale bars, 100 μm.

(G) β-cell mass of CT and KO mice fed an HFD determined as the proportion of the insulin-positive area to the total area of the sections (*p < 0.05; unpaired t test. n = 8).
RESULTS

T-cadherin deficiency alters glucose metabolism under high-fat diet conditions due to attenuated compensative expansion of β-cell mass and insulin insufficiency

We generated systemic T-cadherin knockout mice (T-cadherin^{−−}/; KO) by crossing Cre-terminator mice with control T-cadherin^{flx/flx} mice (CT) in which exon 2 of the Cdh13 (T-cadherin) gene was floxed (Figure S1A). We confirmed that exon 2 in the genomic DNA was deleted (Figure S1A) and that T-cadherin mRNA and its protein were systemically deleted (Figures S1B and S1C). Ectopic accumulation of fat-derived adiponectin was decreased in other tissues (Figure S1C) and plasma adiponectin was increased in KO mice (Figure S1D), as reported previously (Denzel et al., 2010; Matsuda et al., 2015). Body weight and glucose tolerance in 8-week-old mice were not different between CT mice and KO mice (Figures S2A–S2C), nor was pancreatic insulin content different (Figure S2D). Next, we fed CT and KO mice either normal chow (NC) or a high-fat diet (HFD) for 6 weeks (Figure 1A). T-cadherin KO mice gained less body weight than CT mice under HFD conditions mainly due to decreased expansion of white adipose tissues (WATs) (Figures 1B and S3A), as dietary intake was not significantly different (Figure S3B). Glucose tolerance was significantly impaired in KO mice compared to CT mice under HFD conditions but was not significantly different under NC conditions (Figure 1C). Fasting plasma insulin levels were decreased in KO mice compared to CT mice under HFD conditions, and this difference was highly significant 15 min after the glucose challenge (Figure 1D). On the other hand, insulin-induced glucose reduction was not different between the two groups (Figure 1E), and activation of Akt signaling in the insulin-responsive organs was not improved in KO mice despite the decreased body weight and mass of WATs under HFD conditions (Figures S3C–S3F).

Impaired insulin secretion can be caused by decreased β-cell mass or by functional defects in insulin secretion in response to glucose. After 6 weeks of HFD feeding, KO mice displayed reduced β-cell areas in the pancreas and reduced diameter of islets compared to CT mice (Figures 1F–1H). Those were not altered under NC conditions (Figures S3G and S3H). The pancreatic insulin content in KO mice was significantly lower than that of CT mice under HFD conditions (Figure 1I) but was not different under NC conditions (Figure S3I). On the other hand, islets isolated from CT and KO mice similarly secreted insulin in vitro in response to increased glucose levels both under NC and HFD conditions (Figures 1J and S3J).

RNA-sequencing analyses suggest attenuated cell proliferation pathways and Notch signaling in pancreatic β-cells of T-cadherin KO mice

To elucidate the pathological mechanism leading to glucose intolerance in KO mice under HFD conditions, we analyzed the gene expression of isolated islets using RNA sequencing. Principal component analysis indicated that HFD feeding caused the greatest number of differentially expressed genes (DE-Gs) between the islets of CT and KO mice (Figures 2A and S4A–S4D). Among 312 significant DE-Gs in the islets under HFD conditions, 77% were downregulated in KO mice (Figure S4C). Genes associated with the cell cycle and cell proliferation, such as Ccnb1, Cdk1, Pbk, and Mki67, were significantly decreased in the islets of KO mice compared to those of CT mice (Figure 2B). Enriched pathway analyses revealed the downregulation of the pathways of cell cycle and cell proliferation in KO mice under HFD conditions (Figures 2C and 2D). Moreover, genes involved in Notch signaling, such as Dll4, a Notch ligand, Hey1, a Notch target gene, and in the interleukin-6 (IL-6) family, such as Cif1, were significantly decreased in KO mice (Figure 2E). Quantitative PCR analysis confirmed significantly decreased expression of genes involved in cell...
proliferation, Notch signaling (Dll4 and Heyl), and Ctf1 in the islets of KO mice under HFD conditions (Figure 2F). In contrast, genes involved in the maturation of β-cells, such as Ins1, Ins2, Nkx6-1, and Ucn3, were not significantly different (Figure 2G).
Loss of T-cadherin significantly decreases β-cell mass in streptozotocin-induced diabetes

To assess β-cell proliferation under HFD conditions, we measured BrdU incorporation into pancreatic β-cells. KO mice exhibited significantly fewer BrdU-positive β-cells than CT mice (Figure 3A), indicating that T-cadherin deficiency in mice impaired β-cell proliferation under HFD conditions. To further assess the significance of T-cadherin in β-cell proliferation, we challenged 8-week-old CT and KO mice with multiple low-dose streptozotocin (STZ) to induce severe loss of pancreatic β-cells, since this model is typically used to test β-cell survival and adaptive proliferation (Sachs et al., 2020; Tsukita et al., 2017) (Figure 3B). Blood glucose gradually increased after the STZ treatments, and KO mice displayed significantly higher blood glucose and lower pancreatic insulin content than CT mice 18 days after the initial STZ injection (Figures 3C, 3D, and S5A).

T-cadherin expression is not observed in pancreatic β-cells

Next, we examined the expression of T-cadherin in islets. Immunohistochemistry detected only a few very weak T-cadherin signals not overlapping with insulin in both CT and KO mice, suggesting little or no expression of T-cadherin in β-cells (Figure 4A). Weak nonspecific signals in acinar regions were observed in both CT and KO mice. Although the majority of CD31-positive cells were not immunoreactive for T-cadherin, strong T-cadherin signals overlapped with some CD31-positive cells, likely indicating T-cadherin expression in the arteries, as observed in other tissues (Figures 4A and S1C) (Parker-Duffen et al., 2013). Absolutely qPCR detected $3.77 \times 10^{-2}$ copies of Cdh13 (T-cadherin) gene transcripts per Rplp0 transcript in CT islets, which were not significantly different from those of KO islets and far fewer ($3.37 \times 10^{-2}$-fold change) than that of CT aorta (Figure 4B). We further tested T-cadherin expression by western blotting, and it exhibited virtually no signal (Figure 4C). Together, we concluded that T-cadherin was not expressed at a meaningful level in pancreatic β-cells.

Soluble T-cadherin is metabolically regulated and improves β-cell mass and blood glucose levels in streptozotocin-induced diabetes

T-cadherin is a specific binding partner for adiponectin (Kita et al., 2019a). T-cadherin deficiency in mice affected the ectopic accumulation of adiponectin in tissues other than fats (Figure S1C) and increased plasma adiponectin (Figure S1D) as previously reported (Denzel et al., 2010; Fujishima et al., 2017; Nakamura et al., 2020; Obata et al., 2018; Parker-Duffen et al., 2013; Tanaka et al., 2019; Tsugawa-Shimizu et al., 2021). T-cadherin deficiency also reduced EV biogenesis and plasma EV levels similarly to adiponectin deficiency (Nakamura et al., 2020; Obata et al., 2018; Tanaka et al., 2019; Tsugawa-Shimizu et al., 2021). Next, we quantified the plasma EVs in both T-cadherin KO mice and adiponectin knockout (APN-KO) mice after multiple low-dose STZ treatments. As we previously reported (Obata et al., 2018), plasma EVs were similarly decreased in T-cadherin-KO and APN-KO mice compared to CT and wild-type (WT) mice, respectively (Figures S5B and S5C). However, multiple STZ administrations in APN-KO mice did not result in a significant difference in blood glucose or pancreatic insulin content compared to WT mice (Figures S5D–S5F), not phenocopying T-cadherin KO mice (Figures 3C, 3D, and S5A), which suggested adiponectin-independent mechanisms.

Next, we measured soluble T-cadherin levels in the plasma (Fukuda et al., 2021). Our ELISA measures the prodomain-containing 130-kDa form and prodomain-processed 100-kDa form of soluble T-cadherin, but not the 30-kDa prodomain, unlike human plasma (Fukuda et al., 2021). Plasma 130-kDa and 100-kDa soluble T-cadherin were eliminated in T-cadherin KO mice (Figures S1E–S1G). Plasma total (130-kDa + 100-kDa) soluble T-cadherin significantly increased under STZ conditions and mildly decreased under HFD conditions, although the 130-kDa form increased under both conditions (Figures S5A–S5C). Adiponectin deficiency significantly affected the proportion of 130-kDa and 100-kDa forms but did not affect total soluble T-cadherin concentrations (Figures S5G–S5I). Therefore, we hypothesized that total concentrations of soluble T-cadherin might play a role in β-cell proliferation.

We next tested whether soluble T-cadherin could be used to replace whole-body T-cadherin deficiency in the STZ-induced diabetes model (Figure 5D). To this end, we employed a liver-restricted hydrodynamic gene delivery system and introduced the vector encoding T-cadherin without a C-terminal GPI attachment motif. The control vector expressed secreted alkaline phosphatase (SEAP). After STZ treatments, plasma soluble T-cadherin gradually increased in CT mice administered the control vector (CT-SEAP) (Figures 5E, S6A, and S6B). Administration of the soluble T-cadherin vector to KO mice (KO-st-cad) resulted in overexpression of plasma soluble T-cadherin (Figures 5E, S6A, and S6B) and significantly
improved blood glucose levels compared to the administration of the control vector to KO mice (KO-SEAP) (Figures 5F, 5G, and S6C). Pancreatic insulin content was significantly increased in response to the replacement of soluble T-cadherin in KO mice (Figure 5H). The effect of the soluble T-cadherin supplementation was further tested in KO mice under HFD conditions (Figure 5I). Administration of the soluble T-cadherin vector to KO mice (KO-sT-cad) resulted in overexpression of plasma soluble T-cadherin than the control vector to KO mice (KO-SEAP) even after 10 weeks of HFD loading (Figure S5J). The body weight and food intake were not different between the two groups (Figures S6D and S6E). However, the glucose tolerance was partially improved by the soluble T-cadherin vector mice (KO-sT-cad) compared to the control vector to KO mice (KO-SEAP) (Figure 5K), and the proportion of BrdU-positive β-cells was significantly increased by soluble T-cadherin (KO-sT-cad mice) compared to control (KO-SEAP mice) (Figure 5L).

Recombinant soluble T-cadherin upregulates Notch signaling and cell cycle genes in isolated murine islets

Pancreatic β-cell proliferation was reported to be promoted through Notch (Bartolome et al., 2019; Eom et al., 2021), signal transducer and activator of transcription 3 (STAT3) (De Groef et al., 2016; Jimenez-Gonzalez et al., 2013; Miura et al., 2018), and Pbk (Ma et al., 2021) signaling. To evaluate the direct roles

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**Figure 3. Loss of T-cadherin significantly reduces β-cell mass in STZ-induced diabetes**

(A) β-cell proliferation analysis in mice fed an HFD using BrdU incorporation. Representative images of pancreatic sections from CT and KO mice immunostained with insulin and BrdU and the proportion of insulin and BrdU double-positive cells to total insulin-positive cells (**p < 0.01; unpaired t test. n = 6) are shown. Scale bars, 100 μm.

(B) Protocol of the multiple low-dose STZ injections. Mice were intraperitoneally administered 50 mg/kg body weight STZ for 5 consecutive days.

(C) Blood glucose levels of CT and KO mice administered STZ were measured at 9:00 a.m., fed ad libitum (*p < 0.05; unpaired t test. n = 8).

(D) Pancreatic insulin content of CT and KO mice administered STZ was analyzed 18 days after the initial STZ injection (**p < 0.001; unpaired t test. n = 8). Data are shown as the mean ± SEM (A, C, and D). See also Figure S5.
of soluble T-cadherin on islets, isolated murine islets were treated with recombinant soluble T-cadherin (Figure 6A). Recombinant soluble T-cadherin significantly upregulated the expression of the Notch ligands ($\text{Dll1}$ and $\text{Dll4}$) and the targets ($\text{Hes1}$ and $\text{Hey1}$), as well as the cell cycle gene $\text{Ccnb1}$ in the isolated islets of CT mice (Figures 6B–6E and 6G), although the upregulation of gene expressions of the Notch receptor ($\text{Notch1}$) and $\text{Cdk1}$ and $\text{Pbk}$ was not significant (Figures 6F, 6H, and 6I). We next tested whether the Notch signal induction by soluble T-cadherin is required for cell cycle gene induction (Figure 6J). The Notch signal inhibition by N-[N-(3,5-Difluorophenacetyl-L-alanyl)]-(S)-phenylglycine t-butyl ester (DAPT), a small molecule Notch signal inhibitor canceled the upregulated expression of the Notch target ($\text{Hey1}$) and the ligand ($\text{Dll4}$), as well as the cell cycle gene inductions ($\text{Ccnb1}$ and $\text{Cdk1}$) (Figures 6K–6N).

**DISCUSSION**

In this study, we revealed that genetic deficiency of T-cadherin affected glucose metabolism in diet-induced obesity (DIO) due to insufficient pancreatic $\beta$-cell proliferation. Altered glucose metabolism was also evident in STZ-induced diabetes. Soluble T-cadherin improved $\beta$-cell mass and ameliorated glucose
metabolism in STZ-induced diabetes and high-fat diet-induced obesity and upregulated genes associated with Notch signaling and cell cycle in the isolated murine islets. The cell cycle gene induction depended on Notch signaling. We also showed that the expression levels of T-cadherin were negligible in β-cells, contrary to a previous report (Tyrberg et al., 2011). T-cadherin was far less expressed in whole islets than in insulin-responsive tissues such as the muscle, heart, aorta, and other vasculatures which might be the potential sources of soluble T-cadherin in plasma.

The adaptive proliferation of β-cells has been reported to occur through self-duplication of preexisting β-cells in both humans and rodents (Dor et al., 2004; Meier et al., 2008; Teta et al., 2007). Several putative humoral factors have been identified (El Ouaamari et al., 2016; Jimenez-Gonzalez et al., 2013; Rosado-Olivier et al., 2020). However, how insulin-responsive tissues signal the insufficient or sufficiency of insulin to pancreatic β-cells is not understood. In nearly the same STZ-induced diabetes, both cell cycle and apoptosis pathways were reported to be upregulated in β-cells (Tsukita et al., 2017), and transplanted β-cells can adaptively proliferate (Yin et al., 2006). Therefore, certain humoral factors are likely to transmit insulin demands to pancreatic β-cells from insulin-responsive tissues. We showed that the plasma levels of total soluble T-cadherin were increased under insulin deficiency in STZ-induced diabetes and slightly but significantly decreased in diet-induced obesity in CT mice. Thus, total soluble T-cadherin levels in plasma appeared to be inversely related to plasma insulin levels. In diet-induced obesity, although total soluble T-cadherin levels were decreased in CT mice, the complete deficiency of T-cadherin still resulted in poor β-cell proliferation and glucose intolerance, suggesting that soluble T-cadherin is also required for the compensative expansion of β-cell mass under HFD conditions. Therefore, soluble T-cadherin may have a signaling role from T-cadherin-expressing tissues to pancreatic β-cells, working as an endogenous humoral factor required for adaptively increasing β-cell mass in diabetic conditions. Although soluble T-cadherin might also act on other cell types than the pancreatic β-cells, we did not observe any role of soluble T-cadherin on insulin signaling (Figures S6F and S6G) or the adipocyte differentiations (Figure S6H), further suggesting the anti-diabetic role of soluble T-cadherin in the regulation of pancreatic β-cell mass.

Our gene expression analyses suggested that the Notch and IL-6/STAT3 pathways are regulated by T-cadherin in islets. Notch signaling is known to mediate cell fate decisions by interacting with neighboring cells through receptors (Notch 1-4) and ligands (DLL1, 3, 4) and was reported to be involved in the differentiation and proliferation of β-cells (Bartolome et al., 2019; Eom et al., 2021). In addition, cyclin/cyclin-dependent kinase complexes regulate cell cycle progression in β-cells (Kim et al., 2017). Our study reproduced the coordinated down- and upregulation of both effectors of Notch signaling and cyclin B1 in islets in response to a genetic deficiency of T-cadherin in vivo and recombinant soluble T-cadherin in vitro, respectively. Simultaneously, proliferating β-cells marked with BrdU were less
frequently observed in T-cadherin KO mice under HFD conditions, which was significantly rescued by the supplementation of soluble T-cadherin. Our study also indicated that islets from both CT and T-cadherin KO mice expressed comparable levels of genes associated with β-cell maturity under HFD conditions.

Continuous overactivation of Notch signaling by the introduction of a constitutively active Notch intra-cellular domain promoted proliferation but attenuated β-cell maturity (Bartolome et al., 2019). On the other hand, persistent Notch1 inhibition by antisense nucleotides decreased β-cell mass but induced
β-cell differentiation (Eom et al., 2021). These previous reports suggest that Notch signaling promotes β-cell proliferation at the expense of β-cell maturation. In muscle stem cell replication, it was reported that oscillating Notch ligand Dll1 production ensures equilibrium between self-renewal and differentiation (Zhang et al., 2021). Moreover, different Notch ligands activate distinct targets through the same Notch1 receptor by triggering pulsatile or sustained activation dynamics (Nandagopal et al., 2018). Our analyses suggested that soluble T-cadherin increased multiple Notch ligands and their target genes simultaneously, suggesting that dynamic Notch upregulation mechanisms physiologically function to ensure both proliferation and maturation of β-cells. Our analyses also indicated that loss of T-cadherin attenuated the IL-6 family and downstream STAT3 signaling, and the serine/threonine-protein kinase Pbk, whose activities are required for β-cell regeneration and antiapoptosis signaling (De Groef et al., 2016; Jimenez-Gonzalez et al., 2013; Ma et al., 2021; Rosado-Olivieri et al., 2020). Notch is known to signal to STAT3 signaling in different cell types (Kamakura et al., 2004; Matsuno et al., 2018). Signaling through Notch to the IL-6 family/STAT3 pathway and Pbk may be important to ensure both the proliferation and maturation of β-cells.

Because T-cadherin mediates small EV biogenesis and secretion by adiponectin (Kita et al., 2019b; Obata et al., 2018), the lack of T-cadherin in mice reduced plasma EVs in addition to soluble T-cadherin. Small EVs, especially those derived from mesenchymal stem/stromal cells that reside in many tissues, are presumed to exert both protective and regenerative roles (Pittenger et al., 2019). Reduced EV production can be assumed to be partly involved in abnormal glucose handling in T-cadherin KO mice. In this study, we assessed adiponectin deficiency in mice. In APN-KO mice, plasma EV levels were also reduced, but no significant changes were observed in glucose levels or pancreatic insulin content in STZ-induced diabetes. Furthermore, there was no altered gene expression related to the known adiponectin actions such as mitochondrial biogenesis and lipid metabolism in the islets of T-cadherin KO mice under high-fat diet conditions (Figures 2A–2D). These results suggested that EV production by the adiponectin/T-cadherin system (Kita et al., 2019b; Obata et al., 2018) had little effect on β-cell mass.

We distinguishably measured the 130-kDa prodomain-containing form and 100-kDa prodomain-processed form of mouse soluble T-cadherin using our ELISA system. In control mice, while the 130-kDa form was increased in both HFD and STZ conditions, the 100-kDa form and total concentrations of soluble T-cadherin were increased in STZ conditions and slightly decreased in HFD conditions. For the replacement of soluble T-cadherin, we introduced a cDNA coding full-length T-cadherin lacking GPI-anchoring domain at C-terminus into the liver, which resulted in the stable secretion of both 130-kDa and 100-kDa soluble forms of T-cadherin in plasma. The domain requirements of soluble T-cadherin and identification of the receptor for the improvement of β-cell proliferation remain elusive and should be addressed in the future.

In conclusion, our current study uncovered the physiological role of circulating soluble T-cadherin in stimulating β-cell proliferation in diabetes, at least through Notch signaling.

Limitations of the study
Because the receptor of soluble T-cadherin has not been identified, we cannot exclude the possibility that soluble T-cadherin may act on organs other than islets and indirectly affect β-cell mass. Our study revealed a direct signaling ability of recombinant soluble T-cadherin in isolated murine islets. However, the concentration of soluble T-cadherin used herein was greater than physiological levels, which may limit our conclusions. Also, we have not yet determined the precise cell types that secrete soluble T-cadherin or compared their contribution to plasma soluble T-cadherin levels. Identification of the sources of soluble T-cadherin may facilitate our understanding of the pathophysiological roles of soluble T-cadherin on β-cell proliferation.

STAR+METHODS
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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.105404.

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AUTHOR CONTRIBUTIONS

T.O. and S.K. designed the research protocol, performed the biochemical, cellular, and in vivo experiments, analyzed the data, and co-wrote the article. S.F., M.I., and S.K. developed ELISA systems for the measurement of T-cadherin and measured the STZ diabetes-induced increase in soluble T-cadherin in mice. K.F. and E.K-H. assisted in the basic characterization of T-cadherin KO mice. Y.N. and Y.F. assisted with immunofluorescence. D.K. and T.M. assisted with β-cell physiology experiments. H.N. and N.M. contributed to editing the article. S.K. directed and I.S. supervised the project and finalized the article.

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### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Guinea pig monoclonal anti-Insulin | Agilent | Cat#IR-002, RRID: AB_2800361 |
| Rabbit polyclonal anti-Glucagon | Immunostar | Cat#20076, RRID: AB_572241 |
| Rabbit polyclonal anti-CD31 | Abcam | Cat#ab28364, RRID: AB_726362 |
| Goat polyclonal anti-T-cadherin | R&D Systems | Cat#AF3264, RRID: AB_2077121 |
| Rat monoclonal anti-BrdU | Abcam | Cat#ab6326, RRID: AB_305426 |
| Rabbit polyclonal anti-β-Actin | Cell Signaling Technology | Cat#4967, RRID: AB_330288 |
| Rabbit polyclonal anti-α-Tubulin | Cell Signaling Technology | Cat#2144, RRID: AB_2210548 |
| Goat polyclonal anti-Adiponectin | R&D Systems | Cat#AF1119, RRID: AB_2221770 |
| Rabbit polyclonal anti-Phospho Akt (Ser473) | Cell Signaling Technology | Cat#9271S, RRID: AB_10694411 |
| Rabbit polyclonal anti-Akt | Cell Signaling Technology | Cat#9272S, RRID: AB_329827 |
| Rabbit monoclonal anti-Alix | Abcam | Cat#ab186429, RRID: AB_2754981 |
| Rabbit monoclonal anti-Tsg101 | Abcam | Cat#ab125011, RRID: AB_10974262 |
| Rabbit monoclonal anti-Syntenin | Abcam | Cat#ab133267, RRID: AB_11160262 |
| Donkey anti-guinea pig Alexa Fluor 594 | Jackson Immunoresearch Labs | Cat#706-585-148, RRID: AB_2340474 |
| Donkey anti-guinea pig Alexa Fluor 647 | Jackson Immunoresearch Labs | Cat#706-605-148, RRID: AB_2340476 |
| Donkey anti-rabbit Alexa Fluor 555 | Thermo Scientific | Cat#A32794, RRID: AB_2762834 |
| Chicken anti-rat Alexa Fluor 488 | Thermo Scientific | Cat#A21470, RRID: AB_2535873 |
| Goat anti-guinea pig, horseradish peroxidase | Thermo Scientific | Cat#A18775, RRID: AB_2535552 |

| **Chemicals, peptides, and recombinant proteins** | | |
| Streptavidin-horseradish peroxidase | Thermo Scientific | Cat#434323, RRID: AB_2619743 |
| Tyramide reagent Alexa Fluor 488 | Thermo Scientific | Cat#840953 |
| EZ-Link™ Sulfo NHS-LC-LC-Biotin | Thermo Scientific | Cat#A35358 |
| 4',6-diamidino-2-phenylindole (DAPI) | Sigma-Aldrich | Cat#D9542 |
| DAB substrate kit | Thermo Scientific | Cat#34065 |
| Bromodeoxyuridine (BrdU) | Nacalai Tesque | Cat#05650-24 |
| Insulin human | Novo Nordisk | N/A |
| Streptozotocin | Sigma-Aldrich | Cat#S0130 |
| Liberaset TL | Roche | Cat#S01020001 |
| Recombinant soluble T-cadherin | This paper | N/A |
| N-[N-(3,5-Difluorophenacetyl-L-alanyl)]-(S)-phenylglycine t-butyl ester (DAPT) | Nacalai Tesque | Cat#18767-14 |

(Continued on next page)
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Critical commercial assays** |
| Blood glucose-monitoring kit | Sanwa Kagaku Kenkyusho | N/A |
| Mouse insulin ELISA kit | Morinaga Institute of Biological Science | Cat#M1104, RRID_AB_2811268 |
| Mouse total T-cadherin ELISA kit | Immuno-biological Laboratories | This paper |
| Mouse 130-kDa T-cadherin ELISA kit | Immuno-biological Laboratories | This paper |
| Mouse adiponectin ELISA kit | Otsuka Pharmaceutical | Cat#M505652772 |
| Protein assay BCA kit | Nacalai Tesque | Cat#06385-00 |

| Deposited data |
|----------------|
| Raw datasets except for RNA-seq and genomic sequence data | This paper | https://doi.org/10.5061/dryad.qv9s4mgw0 |
| RNA-seq datasets | This paper | GEO: GSE186944 |
| Cdh13 floxed and deleted allele genome sequences | This paper | Cdh13_flox DDBJ: LC733661, Cdh13_deleted DDBJ: LC733662 |

**Experimental models: Cell lines**

| Cell line | Source | Cat# |
|-----------|--------|------|
| HEK293T cells | ATCC | CRL-1573 |
| C2C12 cells | ATCC | CRL-1772 |
| 3T3-L1 cells | ATCC | CL-173 |

**Experimental models: Organisms/strains**

| Organism | Source |
|----------|--------|
| Cdh13(T-cadherin) flox/flox mouse | Transferred from Boston Children’s Hospital |
| E2a-Cre recombinase mouse | Jackson Laboratory |
| Adipoq(Adiponectin)−/− mouse | Obata Y et al., 2018 |

**Oligonucleotides**

| TaqMan Reagent | Source |
|----------------|--------|
| Rplo0 | Thermo Scientific |
| Cdh13 | Thermo Scientific |
| Dll1 | Thermo Scientific |
| Dll4 | Thermo Scientific |
| Hes1 | Thermo Scientific |
| Hey1 | Thermo Scientific |
| Ctf1 | Thermo Scientific |
| Ccnb1 | Thermo Scientific |
| Cdk1 | Thermo Scientific |
| Pbk | Thermo Scientific |
| Mki67 | Thermo Scientific |
| Ins1 | Thermo Scientific |
| Ins2 | Thermo Scientific |
| Nkx6-1 Forward: | This paper |
| Nkx6-1 Reverse: | This paper |
| Nkx2-2 Forward: | This paper |
| Nkx2-2 Reverse: | This paper |
| Ucn3 Forward: | This paper |

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RESOURCE AVAILABILITY

Lead contact
Further information and requests for reagents and resources can be directed to the lead contact, Shunbun Kita (shunkita@endmet.med.osaka-u.ac.jp).

Material availability
All materials and animals in this study are available from the lead contact.
**Data and code availability**

- We submitted all raw datasets except RNA-seq and genomic sequence data to DRYAD with DOI https://doi.org/10.5061/dryad.qv9s4mwwg0. RNA-seq datasets were deposited to NCBI under accession number GEO: GSE186944. The Cdh13 floxed and deleted allele genome sequences were deposited in DDBJ. The GenBank/EMBL/DDBJ accession numbers for the genome sequence of around exon2 of Cdh13; DDBJ: LC733661 for Cdh13_flox, DDBJ: LC733662 for Cdh13_deleted.

- This paper does not report original code.

- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

*Cdh13 (T-cadherin) exon 2flox/flox* mice were transferred from Boston Children’s Hospital under the material transfer agreement. We used the Cre/loxP system to systemically delete T-cadherin. T-cadherin knockout (KO) mice were generated by crossing T-cadherin exon 2flox/flox mice with B6-congenic E2a-Cre transgenic mice (Jackson #003724) to create a germline deletion. Adiponectin knockout (APN-KO) mice were generated previously on the C57BL/6J background (Nakamura et al., 2020). Seven- or eight-week-old male mice were used for the experiments. All animal experiments were performed under 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.

**METHOD DETAILS**

**Diets**

Control T-cadherin*^{flox/flox}* (CT) mice and KO mice were fed either normal chow (NC) (Oriental Yeast) or a high-fat (60 kcal%) diet (HFD) (Research Diets, #D12492) from 8 weeks of age for 6 weeks.

**Metabolic studies**

After feeding NC or a HFD for 6 weeks, we performed an oral glucose tolerance test (OGTT) and insulin tolerance test (ITT). Glucose (2 g/kg body weight) was orally administered after overnight fasting for OGTT. Human insulin (Novo Nordisk; 0.75 U/kg body weight) was intraperitoneally administered after 4 h of fasting for ITT. 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). The plasma concentrations of 130-kDa and total (130-kDa + 100-kDa) soluble T-cadherin were measured using human 130-kDa and total T-cadherin ELISA kits with the standard mouse T-cadherin, respectively (Immuno-Biological Laboratories) (Fukuda et al., 2021). Plasma 100-kDa soluble T-cadherin concentrations were obtained by subtracting the values of 130-kDa soluble T-cadherin concentrations from those of total soluble T-cadherin concentrations. The 30-kDa prodomain form of soluble T-cadherin in mouse plasma cannot be measured in our current ELISA system (Fukuda et al., 2021).

**In vivo insulin signaling assays**

We intravenously administered 0.75 U/kg body weight of human insulin, and 2, 3, and 4 min after the injections, white adipose tissues (WATs), gastrocnemius muscles, and livers were dissected, respectively. We performed western blot analyses of homogenized tissues, and phosphorylated Akt (p-Akt; Cell Signaling Technology, #9271S) and total Akt (Cell Signaling Technology, #9272S) signals were detected.

**Western blotting**

We performed western blot analyses using 4–20% gradient SDS–PAGE (Bio-Rad) and nitrocellulose membranes as previously reported (Obata et al., 2018).

**Pancreatic insulin content**

The dissected pancreases were soaked in 5 mL of 70% ethanol containing 0.18 N hydrochloric acid and homogenized. After overnight incubation at −30°C, the samples were centrifuged (2,000 rpm, 15 min, 4°C), and the supernatants were collected and neutralized with equal volumes of Tris-HCl (pH 7.4). We measured the insulin concentrations of the neutralized samples using the ELISA kit described above and calculated the pancreatic insulin content normalized by the weight of wet pancreatic tissues.
Immunohistochemistry

Pancreases were dissected and incubated in 4% paraformaldehyde for 24 h at 4°C and embedded in paraffin. Paraffinized sections were deparaffinized and processed. Primary antibodies were incubated using the following antibodies: guinea pig anti-insulin (Agilent Technologies, #R-002, 1:2), rabbit anti-glucagon (Immunostar, #20076, 1:100), rabbit anti-CD31 (Abcam, #ab28364, 1:100), rat anti-BrdU (Abcam, #ab6326, 1:250), and goat anti-T-cadherin (R&D, #AF3264, 1:100) conjugated with biotin (Thermo Scientific). Secondary antibodies were incubated using the following antibodies: donkey anti-guinea pig Alexa Fluor 594 (Jackson ImmunoResearch, #706-585-148, 1:400), donkey anti-guinea pig Alexa Fluor 647 (Jackson ImmunoResearch, #706-605-148, 1:400), donkey anti-rabbit Alexa Fluor 555 (Thermo Scientific, #A32794, 1:400), chicken anti-rat Alexa Fluor 488 (Thermo Scientific, #A21470, 1:400), and streptavidin-horseradish peroxidase (Thermo Scientific, #434323, 1:400) followed by tyramide reagent Alexa Fluor 488 (Thermo Scientific, #B40953). Nuclei were stained with DAPI (Sigma–Aldrich). Slides were mounted and imaged using a confocal laser scanning microscope (Olympus, #FV-3000).

β-cell area quantification

The primary antibody (guinea pig anti-insulin, as described above) was incubated, and the secondary antibody (goat anti-guinea pig-horseradish peroxidase, Thermo Scientific) was incubated. After washing, Diaminobenzidine (DAB) staining (Thermo Scientific) was performed according to the manufacturer’s instructions. The relative β-cell area was obtained by calculating the proportion of the DAB-positive area to the total pancreas area using a fluorescence microscope (Keyence, #BZ-710). At least three different sections (at least 200 μm apart from each section) were analyzed and the mean value was calculated for each individual.

Islet isolation

Mice were anesthetized with isoflurane, and an abdominal incision was performed. After clamping the opening of the duodenal papilla, the common bile duct was cannulated using a 30-gauge needle and perfused with Hank’s balanced salt solution (hand-made) containing 0.25 mg/mL Liberase TL (Roche). Pancreases were dissected and digested in 0.25 mg/mL Liberase TL by shaking for 20 min at 37°C. After incubation, 20 mL of RPMI 1640 medium (Sigma–Aldrich, #R1383) containing 10% fetal bovine serum (FBS) and 5.6 mmol/L glucose was added to stop digestion. Centrifugation (300 g for 1 min, 4°C) was performed, and the pellet was suspended in 13 mL of Histopaque-1077 (Sigma–Aldrich, #10771). Then, 10 mL of Dulbecco’s modified Eagle medium (DMEM; serum-free, 5.6 mmol/L glucose, Nacalai Tesque) was gently poured onto the suspension to form a bilayer. Centrifugation (1,500 g for 25 min, 4°C) was performed, and we confirmed that the islets were located between the two layers. The islets were hand-picked using a 1 mL pipette (wide-open tip). After overnight incubation in RPMI 1640 medium containing 10% FBS, penicillin, streptomycin, and 5.6 mmol/L glucose in an incubator (37°C, 5% CO2), the islets were used for experiments.

Islet glucose-stimulated insulin secretion

Islets were handpicked into a tube (10–15 islets per tube) and incubated in 500 μL of KRB (containing 2 mmol/L glucose and 0.1% BSA) for 1 h at 37°C. After discarding the buffer, 500 μL of KRB (containing 2 mmol/L glucose and 0.1% BSA) was added to the tube and incubated for 1 h at 37°C, and the supernatant was collected. Then, 500 μL of KRB (containing 20 mmol/L glucose and 0.1% BSA) was added to the tube and incubated for 1 h at 37°C, and the supernatant was collected. Finally, 30 μL of RIPA buffer with protease inhibitor (Nacalai Tesque) was added to the tube and vortexed. The proportion of the insulin content in the supernatant to the intra-islet insulin content was calculated.

RNA-sequences

The qualities of the total RNA extracts were analyzed using an RNA 6000 Pico Kit (Agilent Technologies), and we confirmed that the samples for RNA-seq analysis had RNA integrity numbers of 8.0 or more. RNA samples were sequenced on a HiSeq 2500 (Illumina), and data of raw count and fragments per kilobase of exon per million mapped reads (FPKM) were obtained. The data were loaded into the iDEP ver. 0.94 web application. Gene expression analysis was conducted using the R package DESeq2 method; differentially expressed genes (DEGs) between KO mice islets and CT mice islets were determined based on false discovery rate (FDR) < 0.05 and |log2 fold change| ≥ 1. Enriched pathway analyses were performed using Gene Ontology (GO) biological process and Ingenuity Pathway Analysis (IPA) canonical pathway (QIAGEN).
Quantitative PCR (qPCR) analyses were performed using a Quantstudio 7 Real-Time PCR System (Thermo Scientific). PCR primers or TaqMan Gene Expression Assay reagents (Thermo Scientific) were used. For mRNA expression analyses, the Ct values were transformed to the linear expression values that were normalized to the reference gene (Rplp0) and the control group. In TaqMan Gene Expression Assays, we used the ∆∆Ct method (the Rplp0 gene was used as the reference). The absolute quantification of Cdh13 mRNA transcripts was performed using Cdh13 and Rplp0 cDNA standards whose concentrations were already calculated.

BrdU incorporation
Mice were intraperitoneally administered BrdU (Nacalai Tesque; 50 mg/kg body weight) for 4 consecutive days. Four hours after the last injection, the pancreas was dissected. We performed immunostaining insulin and BrdU with the antibodies as described above, and the proportion of insulin and BrdU double-positive cells to the total insulin-positive cells was calculated.

Streptozotocin treatment
Streptozotocin (STZ) (Sigma–Aldrich) dissolved in 0.1 mol/L citrate buffer was intraperitoneally injected into 8-week-old male mice at 50 mg/kg for 5 consecutive days. After the treatment, blood glucose was measured twice per week at 9:00 AM, and mice were fed ad libitum.

Isolation of plasma extracellular vesicles
Plasma samples were mixed with thrombin (500 U/mL) for 10 min to remove fibrin, followed by centrifugation at 12,000 g for 20 min. For extracellular vesicle (EV) isolation, the samples were ultracentrifuged at an average of 110,000 g for 2 h, followed by a washing step of the exosome pellet with Dulbecco’s phosphate-buffered saline with calcium and magnesium [PBS (+)] at an average of 110,000 g for 2 h (TLA100.1 rotor, Beckman Coulter). The EV pellets were directly solubilized in the Laemmli sample buffer (Obata et al., 2018). We quantified the amount of EVs by western blotting using the following antibodies; rabbit anti-Alix (Abcam, #ab186429), rabbit anti-Tsg101 (Abcam, #ab125011), and rabbit anti-Syntenin (Abcam, #ab133267).

Hydrodynamic gene delivery
The cDNA coding the Cdh13 (T-cadherin) gene (NM_019707) without a C-terminal GPI-anchoring site (2079 bp) was cloned into the pLIVE vector (Mirus) to establish the vector for soluble T-cadherin. We used secreted embryonic alkaline phosphatase (SEAP) as a control vector (Mirus). The vectors were dissolved into TransIT-QR Hydrodynamic Delivery Solution (Mirus), and 500 mg/kg body weight of plasmids was intravenously injected into the tail vein within 3 seconds according to the manufacturer’s instructions.

Recombinant soluble T-cadherin treatment to isolated islets
The cDNA coding soluble T-cadherin (as described above) was cloned into pcDNA3.1(+) and transiently transfected into human embryonic kidney 293T cells. The conditioned medium without serum supplementation was collected, and soluble T-cadherin was purified as previously described (Fukuda et al., 2021). Islets isolated from 14-week-old CT mice were incubated in an RPMI medium containing 5.6 mmol/L glucose for 24 h at 37°C. After incubation, 10 islets were handpicked, placed into a tube, and incubated in an RPMI medium containing 11 mmol/L glucose and 0, 7.7, or 77 nmol/L recombinant soluble T-cadherin for 1 or 24 h at 37°C. The concentration of recombinant soluble T-cadherin was determined based on the BCA protein assay (Nacalai Tesque). To evaluate whether Notch signaling was involved, DAPT dissolved in dimethyl sulfoxide at 50 mmol/L was added to the incubation media at the final concentration of 50 µmol/L (1:1,000 dilution). As the control, the equal volume of dimethyl sulfoxide was added to the media. The islets were incubated for 24 h at 37°C.

Recombinant soluble T-cadherin treatment to C2C12 cells and 3T3-L1 adipocytes
Cells were differentiated with DMEM high glucose containing 1% FBS for four days. Following two times of washing with 1-mL serum-free DMEM, the cells were incubated with or without 7.7 nmol/L soluble T-cadherin in serum-free DMEM containing 0.2% (w/v) bovine serum albumin (Sigma–Aldrich) for 4 h. Insulin was added to the incubation media at a final concentration of 100 nmol/L and incubated for 30 min. The Akt phosphorylation (Cell Signaling Technology, #9271) was evaluated by western blotting and the relative
phosphorylation to total Akt (Cell Signaling Technology, #9272) was quantified. 3T3-L1 preadipocyte cells were differentiated with DMEM high glucose containing 10% FBS, 1.0 μmol/L dexamethasone, 0.5 mmol/L IBMX, and 1.0 μg/mL bovine insulin for 3 days. The cells were cultured with DMEM high glucose containing 10% FBS and 1.0 μg/mL bovine insulin for additional 3 days. Following two times of washing with 1-mL serum-free DMEM, the cells were incubated with or without 7.7 nmol/L soluble T-cadherin in serum-free DMEM containing 0.2% (w/v) bovine serum albumin for 4 h. Insulin was added to the incubation media at a final concentration of 100 nmol/L and incubated for 30 min. The Akt phosphorylation was evaluated by western blotting and the relative phosphorylation to total Akt was quantified. Oil Red O Staining was used for measuring stored lipids of the mature adipocytes. After 6 days of adipocyte differentiation with or without soluble T-cadherin, 3T3-L1 adipocytes were washed with PBS and fixed with 10% formalin for 30 min. Formalin was removed and the cells were gently washed twice with PBS followed by a 5-min incubation in 60% isopropanol. The 60% isopropanol was aspirated, and 1 mL of Oil Red O solution (Sigma–Aldrich, #O1391) was added evenly over the cells in each well and incubated for 10 min. After removing the Oil Red O solution, the cells were extensively washed with sterile water before acquiring the images. Oil Red O dye was also eluted with hexane/isopropanol (3:2), dried up, and solubilized in isopropanol. The OD was measured at 490 nm for quantification.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

We performed statistical analyses using JMP Pro 15 (SAS Institute). The p values less than 0.05 were considered statistically significant. The results are expressed as the mean ± SEM from at least three independent biological experiments unless otherwise specified. The methods of statistical tests and sample sizes are provided in the figure legends.