Inactivation of the dual Bmp/Wnt inhibitor Sostdc1 enhances pancreatic islet function

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Henley KD, Gooding KA, Economides AN, Gannon M. Inactivation of the dual Bmp/Wnt inhibitor Sostdc1 enhances pancreatic islet function. Am J Physiol Endocrinol Metab 303: E752–E761, 2012. First published July 24, 2012; doi:10.1152/ajpendo.00531.2011.—Current endeavors in the type 2 diabetes (T2D) field include gaining a better understanding of extracellular signaling pathways that regulate pancreatic islet function. Recent data suggest that both Bmp and Wnt pathways are operative in pancreatic islets and play a positive role in insulin secretion and glucose homeostasis. Our laboratory found the dual Bmp and Wnt antagonist Sostdc1 to be upregulated in a mouse model of islet dysmorphogenesis and nonimmune-mediated lean diabetes. Because Bmp signaling has been proposed to enhance β-cell function, we evaluated the role of Sostdc1 in adult islet function using animals in which Sostdc1 was globally deleted. While Sostdc1-null animals exhibited no pancreas development phenotype, a subset of mutants exhibited enhanced insulin secretion and improved glucose homeostasis compared with control animals after 12 wk exposure to high-fat diet. Loss of Sostdc1 in the setting of metabolic stress results in altered expression of Bmp-responsive genes in islets but did not affect expression of Wnt target genes, suggesting that Sostdc1 primarily regulates the Bmp pathway in the murine pancreas. Furthermore, our data indicate that removal of Sostdc1 enhances the downregulation of the closely related Bmp inhibitors Ctgf and Gremlin in islets after 8-wk exposure to high-fat diet. These data imply that Sostdc1 regulates expression of these inhibitors and provide a means by which Sostdc1-null animals show enhanced insulin secretion and glucose homeostasis. Our studies provide insights into Bmp pathway regulation in the endocrine pancreas and reveal new avenues for improving β-cell function under metabolic stress.

Type 2 diabetes; β-cell function; sclerostin domain-containing 1; Bmp; Wnt

Type 2 diabetes (T2D) results from a loss of insulin sensitivity in the peripheral tissues and a decline in functional pancreatic β-cell mass. The β-cells of the pancreas secrete insulin in response to subtle changes in blood glucose and, along with other islet endocrine cells, are responsible for maintaining glucose homeostasis within the organism. Typically, treatments for T2D target one area of impaired glucose homeostasis, such as improving insulin sensitivity in peripheral tissues, decreasing hepatic glucose output, or increasing insulin secretion. For example, sulfonylureas, insulin secretagogues that act at the level of the voltage-gated potassium (K_ATP) channel on β-cells, initially improve glycemia by increasing insulin secretion. However, over time sulfonylureas lose efficacy and β-cells become exhausted; eventually patients require supplemental treatments to maintain glucose homeostasis before exogenous insulin therapy is required (6).

Recently, extracellular modulation of pancreatic islet function has become an area of study that is especially promising to the development of novel T2D therapeutics that improve more than one area of glucose homeostasis. Increasing evidence suggests that manipulation of bone morphogenetic protein (Bmp) and Wingless-type MMTV integration site family (Wnt) signaling in pancreatic islets is beneficial to islet function and β-cell proliferation (8, 11, 12). The Bmp signaling pathway is activated upon ligand binding to Bmp-type 1 and 2 receptors, followed by autophosphorylation of the receptors and subsequent activation of downstream receptor (R) Smad1, -5, and -8 proteins. R-Smads form a complex with the ubiquitously expressed co-Smad Smad4 and translocate to the nucleus to mediate transcription of target genes. In the pancreas, ablation of Bmp receptor 1a (Bmpr1a) in β-cells or expression of dominant negative Bmpr1a in the pancreatic epithelium leads to glucose intolerance, decreased expression of genes involved in glucose sensing and metabolism, and decreased insulin production and secretion (5, 20). Conversely, Pdx1-mediated overexpression of Bmp4 throughout the pancreatic epithelium improves glucose tolerance and insulin secretion and increases expression of genes involved in islet function (5).

Canonical Wnts bind to the Frizzled (Fzd) and lipoprotein receptor-related protein (LRP)-5/6 coreceptors, ensuing in dissociation of the cytoplasmically located β-catenin “degradation complex”, which is composed of adenomatosis polyposis coli (APC), axin, and the serine-threonine kinase glycogen synthase kinase (Gsk)-3β. Dissociation of this complex enables the stabilization and nuclear localization of β-catenin, which facilitates transcription of Wnt target genes. In vivo studies have shown that global loss of the Wnt coreceptor Lrp5 causes glucose intolerance and impaired insulin secretion in mice (4). Activation of Wnt signaling in pancreatic islets by exendin 4 (Ex4), the glucagon-like peptide-1 (GLP1) agonist and current T2D therapy, or with Wnt ligands induces expression of cell cycle activators cyclin D1 and Cdk4, respectively (11). Furthermore, β-cell-specific loss of Gsk-3β results in expanded β-cell mass and resistance to diet-induced diabetes (13). Overall, the data show that both Bmp and Wnt signaling activities play a positive role in islet function and β-cell proliferation, and this burgeoning field of study holds promise for developing new types of T2D treatment.

The majority of the literature surveying Bmp and Wnt signaling in adult islet function focuses mainly on manipula-
tion of pathway ligands or receptors. To date, few studies have investigated the role of extracellular Bmp and/or Wnt antagonists in adult pancreatic β-cell function. Our laboratory identified an almost twofold upregulation of sclerostin domain-containing 1 (Sostdc1; also referred to as WISE, USAG-1, ectodin), a dual Bmp/Wnt inhibitor, in postnatal day (P)1 pancreata from transgenic mice misexpressing hepatocyte nuclear factor (Hnf)6 in islet endocrine cells. The Hnf6 transgenic mouse is a model of non immune-mediated lean diabetes (23, 25). Sostdc1 belongs to the eight-ring cysteine knot family of Bmp inhibitors and binds Bmp ligands directly to antagonize signaling (9, 10). Similarly, Sostdc1 interacts with the cysteine knot structure of LRP6 to antagonize Wnt signaling, and has been shown to preferentially inhibit this pathway in other cell systems (10).

Bmp/Wnt inhibitors such as Sostdc1-LacZ were generated as described previously (14). Sostdc1LacZ/LacZ plug was considered embryonic day (e)0.5. All mouse studies were performed in accordance with the Vanderbilt Institutional Animal Care and Use Committee guidelines under the supervision of the Division of Animal Care.

**Tissue preparation and histology.** Animals were fasted for 12:12-h light-dark cycle and received food and water ad libitum. Animals received a regular chow diet (11% kcal from fat, LabDiet, no. 5L15) or HFD (60% kcal from fat, Bio-Serv, no. S3282) at 4 wk of age for 4, 8, or 12 wk. For embryonic studies, the presence of a vaginal plug was considered embryonic day (e)0.5. All mouse studies were performed in accordance with the Vanderbilt Institutional Animal Care and Use Committee guidelines under the supervision of the Division of Animal Care.

**Materials and Methods**

*Generation of animals and husbandry.* Sostdc1-homozygous (Sostdc1+/+) animals were generated as described previously (21, 26). Briefly, a cytoplasmic lacZ allele was used to replace the entire open reading frame of Sostdc1 and generate a knock-in allele. BRE-LacZ transgenic animals were generated as described previously (14). Animals were housed on a 12:12-h light-dark cycle and received food and water ad libitum. Animals received a regular chow diet (11% kcal from fat, LabDiet, no. 5L15) or HFD (60% kcal from fat, Bio-Serv, no. S3282) at 4 wk of age for 4, 8, or 12 wk. For embryonic studies, the presence of a vaginal plug was considered embryonic day (e)0.5. All mouse studies were performed in accordance with the Vanderbilt Institutional Animal Care and Use Committee guidelines under the supervision of the Division of Animal Care.

**X-Gal staining.** Embryonic tissues and adult pancreata were dissected in cold 1× PBS and fixed in 4% paraformaldehyde at 4°C for 2 h (embryos) and 4 h (adults). Tissues were permeabilized for 15–30 min in 2 mM MgCl2–0.01% sodium deoxycholate-0.02% Nonidet P-40 in 1× PBS and reacted with 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-Gal) substrate solution overnight at room temperature.

**Genotyping.** Genotyping for the following alleles was performed by PCR using the primers listed: Sostdc1: WT F: CCTTCTCTGTGTGTTTCACTCCG; WT R: TGATTCAGGTGTCGTTTG; LacZ R: CCGTAATGGGATAGGTCACG (15); BRE-LacZ: BRE-LacZ F: CCTTCTCGTATTACCCAG; BRE-LacZ R: TTAAGTTGGGTAGAGAGAACTCTT.

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anti-glucagon (Linco, 1:1,000), and rabbit phospho-histone H3 (PH3; Millipore, 1:200) and were incubated at 4°C overnight. Secondary antibodies (Jackson ImmunoResearch, 1:300) were incubated for 1 h at room temperature.

**Analysis of β-cell mass.** Sections ~250 μm apart (10 sections per animal) were immunolabeled for insulin followed by a peroxidase-conjugated secondary antibody, visualized using a DAB Peroxidase Substrate Kit (Vector Laboratories), and counterstained with eosin. Digital images were generated using a Nikon Coolscan 9000 and NikonScan software (v. 4.0.2). Total pancreatic and insulin-positive areas of each section were measured using MetaMorph Software (v. 7.7, Molecular Devices). β-Cell mass was calculated by the ratio of insulin-positive area to total pancreas area of all sections for each animal multiplied by the tissue wet weight.

**Analysis of β-cell proliferation.** Sections 600 – 900 μm apart (3 sections per animal) were colabeled for insulin and PH3 followed by species-specific Cy2- or Cy3-conjugated secondary antibodies. The PH3 primary antibody required antigen retrieval in 10 mmol/l sodium citrate, pH 6.5, followed by 0.2% Triton X-100 in PBS. Nuclei were labeled with 1.5 g/ml 4’,6-diamidino-2-phenylindole (DAPI, Molecular Probes) in mounting medium. All insulin-positive cells on each section were imaged at ×400 magnification on an Olympus BX41 microscope with a digital camera using Magnafire (Optronics). A minimum of 3,000 β-cells per animal were counted. Percent proliferating β-cells equaled the number of insulin/PH3 double-positive cells divided by the total number of insulin-positive cells.

**RNA isolation and real-time quantitative PCR.** RNA from freshly isolated islets (75–125 islets per mouse) was extracted and DNase treated using the RNAqueous and Turbo DNA-free kits (Ambion). RNA quality and quantity were assessed using an NF-1000 spectrophotometer (NanoDrop) and a 2100 electrophoresis bioanalyzer (Agilent). cDNA was generated from 50 ng of RNA using the Superscript III First-Strand Synthesis System for RT-PCR (Invitrogen). Real-time PCR was performed using the iQ5 Real-Time PCR Detection System (Bio-Rad) with the Real-Time SYBR Green PCR Supermix (Bio-Rad) and primers designed to detect specific genes. Results were quantified using the 2^ΔΔCt method (27). Primers and PCR conditions are available upon request.

**Statistical analysis.** Insulin secretion data were measured using one-way ANOVA and IPGTT by two-way ANOVA with Bonferro-ni’s posttest. Statistics for area under the curve were measured using one-way ANOVA. Gene expression, β-cell mass, and proliferation data were analyzed by Student’s t-test using GraphPad Prism (v. 5.01). A value of P < 0.05 was considered significant.

**RESULTS**

**Sostdc1 expression in the developing and adult pancreas.** To evaluate the expression pattern of Sostdc1 in the developing and adult pancreas, pancreata were dissected from Sostdc1LacZ/ animals at e14.5, e18.5, and 8 wk of age and assayed for β-galactosidase (β-Gal) activity by X-Gal staining. At all time points evaluated, β-Gal activity was restricted to the acinar component of the exocrine tissue and excluded from the islets as demarcated by insulin staining (Fig. 1, A and B, and data not shown). Additionally, we assessed Sostdc1 expression by β-Gal activity in insulin-sensitive tissues. X-Gal staining was not detected in adipose tissue, liver, insulin-responsive regions of the brain, cardiac muscle, or skeletal muscle from Sostdc1 heterozygous animals (data not shown). Sostdc1 mutants showed no defects in pancreas or islet development or adult islet morphology (Fig. 1, C and D, and data not shown).

**Insulin secretion is enhanced in a subset of Sostdc1-null mutant animals.** To explore the hypothesis that loss of a dual Bmp and Wnt antagonist would enhance islet function and glucose homeostasis, we subjected control and Sostdc1-null animals to islet perifusions and IPGTTs. As shown below, Sostdc1-null animals showed a divergent phenotype. Ex vivo insulin secretion was assessed in islets from animals exposed to HFD for 8 wk using islet perfusion. Islets isolated from a subset of Sostdc1-null animals exhibited significantly greater basal insulin secretion (>2.00 ng·100 EQs⁻¹·min⁻¹) and secreted significantly more insulin in response to 16.7 mM glucose compared with control animals and Sostdc1-null animals with basal insulin secretion ≤1.00 ng·100 EQs⁻¹·min⁻¹ (Fig. 2A). In control and KO animals with lower basal insulin secretion (≤1.00 ng·100 EQs⁻¹·min⁻¹), administration of 16.7 mM...
glucose stimulated an 11.3-fold and 8.3-fold increase in insulin secretion compared with basal (5.6 mM glucose) insulin secretion, respectively. KO animals with elevated basal insulin secretion (>2.00 ng·100 EQs⁻¹·min⁻¹) demonstrated a 4.86-fold increase in insulin secretion (Fig. 2A). Despite the reduced fold change in insulin secretion in response to elevated glucose, the Sostdc1-null mutants with raised basal insulin secretion showed a threefold increase in insulin release overall in

**Fig. 2.** Insulin secretion from islets from Sostdc1-null animals is enhanced after exposure to high-fat diet (HFD). A: islets from a subset of Sostdc1-null animals showed significantly greater basal insulin secretion (>2.00 ng·100EQs⁻¹·min⁻¹) and secreted significantly more insulin when stimulated with glucose compared with control islets and null islets with low basal insulin secretion (<1.00 ng·100EQs⁻¹·min⁻¹). Error bars represent SE. Animals were exposed to HFD for 8 wk. B: quantification of area under the curve for A. Means ± SE: Control basal <1.00: 15.02 ± 3.037; KO basal >2.00: 61.60 ± 8.102. For A and B Control (basal <1.00 ng·100EQs⁻¹·min⁻¹), n = 6; KO (basal <1.00 ng·100EQs⁻¹·min⁻¹), n = 6; KO (basal >2.00 ng·100EQs⁻¹·min⁻¹), n = 5. C: glucose homeostasis in control (n = 3) and Sostdc1-null (n = 5) animals after 4-wk chow diet. D: subset of Sostdc1-null animals with fasting blood glucose (FBG) <125 mg/dl exhibit significantly improved glucose homeostasis compared with Control and KO animals with FBG >125 mg/dl during an intraperitoneal glucose tolerance test. WT (FBG >125 mg/dl, n = 6); WT (FBG <125 mg/dl, n = 11); KO (FBG >125 mg/dl, n = 10); KO (FBG <125 mg/dl, n = 8). Animals were exposed to HFD for 12 wk. Error bars represent SE. KO (FBG <125 mg/dl) vs. KO (FBG >125 mg/dl): *P < 0.05, **P < 0.01, ***P < 0.001. KO (FBG <125 mg/dl) vs. Control (FBG >125 mg/dl): #P < 0.05 , ##P < 0.01. E: insulin tolerance tests in control (n = 2) and Sostdc1 KO animals (n = 5) after exposure to HFD for 12 wk. Error bars represent SE.
response to high glucose compared with the other two groups (Fig. 2B).

To evaluate whether loss of Sostdc1 improves in vivo glucose homeostasis, IPGTTs were performed on chow-fed control and Sostdc1-null animals at 4 and 8 wk of age. Under these conditions, there were no discernible differences in glucose homeostasis between genotypes (Fig. 2C and data not shown). These results are consistent with data showing that mice lacking Sostdc1 do not exhibit changes in β-cell proliferation or mass on a chow diet (Fig. 3, A and B), nor was there any difference in overall pancreas weight (data not shown). To determine whether loss of Sostdc1 improves insulin secretion and glucose homeostasis in the setting of increased metabolic demand, animals were exposed to HFD for 8 or 12 wk and IPGTTs were performed at both time points. We observed that control and Sostdc1 animals split into two groups based on fasting blood glucose (FBG): FBG < 125 mg/dl or FBG > 125 mg/dl (Fig. 2D and data not shown; see Table 1 for average FBG for each group). We were unable to correlate a decrease in FBG with improved basal insulin secretion in the islet perfusion assay. Interestingly, after 8 or 12 wk of HFD, Sostdc1-KO animals with FBG of <125 mg/dl showed significantly improved glucose homeostasis compared with KO animals with FBG of >125 mg/dl and control animals with FBG of >125 mg/dl (Fig. 2C and data not shown). However, there were no differences in glucose homeostasis between control animals of either FBG level (Fig. 2D). We did not attribute changes in FBG or glucose homeostasis to altered insulin sensitivity, as there were no changes in these parameters between genotypes after 12-wk HFD feeding (Fig. 2E). These data agree with the fact that Sostdc1 expression was not observed in key insulin-responsive tissues.

Importantly, any time low FBG was observed in a KO animal, this animal always showed improved glucose clearance throughout the IPGTT. Likewise, any time islets isolated from a KO animal showed increased basal insulin secretion, these islets always showed an enhanced insulin-secretory response to elevated glucose in the perfusion assay. Additionally, no changes in β-cell mass or proliferation were observed after 12-wk HFD feeding (Fig. 3, C and D), supporting the hypothesis that changes observed in Sostdc1-null animals are related to β-cell function and not increased β-cell mass.

**Assessment of genetic background in Sostdc1-null animals.** Investigation of insulin secretion and glucose homeostasis revealed a divergent phenotype in Sostdc1-null animals. In particular, we observed a deviation in basal insulin secretion from isolated islets after exposure to HFD for 8 wk (Fig. 2, A and B) and in FBG after exposure to HFD for 12 wk (Fig. 2D). These data suggest that in a subset of mutant animals the loss of Sostdc1 is beneficial to β-cell function. The Sostdc1-null

**Table 1. Average fasting blood glucose values after exposure to HFD for 12 wk**

<table>
<thead>
<tr>
<th></th>
<th>WT &lt;125 mg/dl</th>
<th>WT &gt;125 mg/dl</th>
<th>KO &lt;125 mg/dl</th>
<th>KO &gt; mg/dl</th>
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<tbody>
<tr>
<td>Average fasting glucose</td>
<td>94.0 ± 4.70</td>
<td>172.3 ± 17.02</td>
<td>91.25 ± 6.71</td>
<td>152.0 ± 8.36</td>
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Values are means ± SE in mg/dl. HFD, high-fat diet; WT, wild type; KO, Sostdc1 knockout.
animals used in these experiments were of mixed genetic background, consisting of C57Bl/6, DBA, and 129Sv. To determine whether the divergent phenotype could be attributed to the varying contribution of these different genetic backgrounds, we utilized genome scanning analysis to identify background-specific genetic markers that might be correlated with the improved phenotype (17). To this end, tissue samples were collected from control and KO animals and analyzed for SNP markers associated with C57Bl/6, DBA, and 129Sv genetic backgrounds. It has been well established that the C57Bl/6 strain is prone to impaired glucose tolerance and insulin secretion with age due to a mutation within the nicotinamide nucleotide transhydrogenase (Nnt) gene (22). Thus, we predicted that loss of Sostdc1 would improve insulin secretion in animals predisposed to β-cell dysfunction and anticipated that null animals showing the improved phenotype would also show higher C57Bl/6 genetic contribution. Interestingly, genome scanning analysis did not reveal any striking variations in composition of genetic background within Sostdc1-null animals. Indeed, of the mutant animals assessed, contribution of C57Bl/6 to genetic background consistently ranged from 44.3 to 54.49%, indicating that broad differences in genetic background do not play a role in the divergent phenotype of Sostdc1-null animals. In addition, we assessed whether an increased number of Nnt mutant alleles in a given animal was associated with improved glucose homeostasis in the absence of Sostdc1. However, we found that the presence of this mutant allele could not account for the observed phenotype (data not shown).

Analysis of Bmp signaling activity using the BRE-LacZ transgene. As shown in Fig. 1, we did not observe β-Gal activity in the islets of the Sostdc1LacZ/+ animals. To evaluate whether Bmp signaling activity is increased in islets in the absence of Sostdc1, we bred animals carrying the Bmp response element (BRE)-LacZ transgenic reporter allele (14) with animals lacking Sostdc1. We predicted that elevated Bmp signaling in the absence of Sostdc1 would result in activation of the BRE-LacZ transgene (and thus X-Gal staining) in islets. Islets from chow-fed Sostdc1 KO animals did not exhibit increased phosphorylated Smad1, a readout of active Bmp signaling (Fig. 4), consistent with the finding that unstimulated Sostdc1 KO animals did not display basal improvements in glucose homeostasis. We hypothesized that loss of this dual Bmp and Wnt inhibitor would enhance islet Bmp signaling in the setting of metabolic stress, since we did observe improved β-cell function and insulin secretion in animals on HFD. After exposure to HFD for 4 wk, Sostdc1-null animals carrying the BRE-LacZ transgene and control animals (BRE-LacZ transgene or Sostdc1 KO alone) were euthanized, and pancreata were harvested for analysis of β-Gal activity. We did not detect β-Gal activity in the islets of any animals exposed to HFD (data not shown; n = 4 for BRE-LacZ; n = 2 for BRE-lacZ, Sostdc1 KO) but X-Gal staining of the acinar tissue was observed in Sostdc1 mutant animals (data not shown). X-Gal staining was detected in the kidneys of animals carrying the BRE-LacZ transgene, as reported by others (2), indicating that the transgene is operative in Bmp-responsive tissues in our hands (data not shown).

Loss of Sostdc1 alters expression of Bmp-responsive genes and Bmp and Wnt signaling modulators. As described above, both Bmp and Wnt signaling positively regulate β-cell function and proliferation, and we hypothesized that loss of a dual Bmp/Wnt antagonist would enhance activity of either or both of these pathways in islets. Because it is unclear whether Sostdc1 preferentially inhibits Bmp or Wnt signaling in islets, and because both pathways are operative in β-cells, we assessed expression of selected ligands and target genes from both pathways. Bmp target genes were chosen based on previous work indicating their importance to glucose homeostasis and insulin secretion (5), and specific Wnt target genes were evaluated due to their known roles in cell cycle regulation and pancreas development (10, 11, 13, 21). Gene expression was assessed at multiple time points using qRT-PCR on islets isolated from control and Sostdc1-null animals after exposure to chow and HFD. No changes in expression of any of the selected genes were detected between chow-fed control and mutant islets isolated from animals at 8 wk of age (Fig. 5A). Loss of Sostdc1 resulted in significantly increased expression of the glucose transporter (Glut2) after exposure to HFD for 4 wk (8 wk of age; Fig. 5B). At this time point, we also observed a trend toward decreased expression of Nkx6.1, Id2, peroxisome proliferator-activated receptor (PPAR)6, c-Myc, matrix metalloproteinase (MMP)-7, and connective tissue growth factor (CTGF) in islets isolated from Sostdc1 mutant mice (Fig. 5B). Additionally, mutant islets showed a trend toward increased expression of Bmp7 and Bmpr1a after 4 wk of HFD exposure. Extension of HFD exposure to 8 wk (12 wk of age) did not result in a statistical change in Wnt-responsive gene expression (Fig. 5C). However, significant downregulation in expression of Smad1 and Nkx6.1, both of which are Bmp-regulated in β-cells (5), was observed in the absence of Sostdc1 (Fig. 5C) compared with control animals, islets from animals lacking Sostdc1 exhibited significantly reduced expression of other Bmp and Wnt pathway modulators that are structurally and functionally similar to Sostdc1, including Gremlin and Ctgf. However, we did not observe a significant change in expres-
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**Fig. 5.** Gene expression in Sostdc1-null islets on chow diet or HFD. Expression of specific Bmp and Wnt ligands, pathway-responsive genes, and Bmp/Wnt modulators was assayed using quantitative real-time PCR in islets isolated from control or Sostdc1-null animals on chow diet for 4 wk (A), HFD for 4 wk (B), or HFD for 8 wk (C). Expression levels were normalized against ubiquitously expressed Hprt. Error bars represent SE. *P < 0.05, **P < 0.01, ***P < 0.001; n ≥ 3 for each genotype.

**Fig. 6.** Deletion of Sostdc1 during HFD stimulation reduces expression of other Bmp and Wnt modulators. Expression of Sostdc1, Ctgf, and Gremlin was assayed using quantitative real-time PCR in islets isolated from control or Sostdc1-null animals after 8 wk of chow or HFD. Expression levels were normalized against ubiquitously expressed Hprt. Error bars represent SE. *P < 0.05, **P < 0.01, ***P < 0.001; n ≥ 3 for each genotype.

DISCUSSION

Our studies have explored the modulation of Bmp and Wnt signaling in adult islet function, an area of study that still requires additional characterization. By utilizing animals globally lacking the dual Bmp and Wnt inhibitor Sostdc1, we evaluated the role of this protein in insulin secretion and glucose homeostasis. Current literature indicates that both Bmp and Wnt positively regulate components of these pathways, either globally or in a tissue-specific manner, impairs β-cell function, insulin secretion, and glucose homeostasis (4, 6, 7, 17, 18). The Hnf6 misexpression model generated by our group exhibits defects in insulin secretion that are reminiscent of those observed in Bmpr1a and Lrp5 mutant animals (2, 3, 8, 10). Concordantly, in Hnf6-misexpressing animals there is a twofold upregulation of Sostdc1, a dual Bmp and Wnt inhibitor that interacts with both Bmp4 and the LRP6 receptors (11). Taken together, these data suggest that irregular modulation of Bmp and/or Wnt signaling may contribute to impaired insulin secretion observed in some mouse models of diabetes.

The detection of Sostdc1-driven β-Gal activity in the acinar tissue, but not the islets, of Sostdc1-null animals implies that Sostdc1 may be secreted from acinar tissue to affect islet function. To date, no studies have indicated a role for Bmp or Wnt inhibitors in pancreatic acinar tissue. However, we were able to quantify a decrease in expression of endogenous Sostdc1 in adult islets exposed to HFD compared with control chow (Figs. 5 and 6), suggesting that Sostdc1 is indeed expressed in islets but at levels too low to detect by X-Gal staining.

We saw no alterations to pancreas development in Sostdc1-null animals in terms of endocrine cell number or islet morphology. This observation is consistent with the previously published model of Bmp4 overexpression in the pancreatic...
epithelium [Pdx1-Bmp4, (5)], which did not exhibit a developmental phenotype. Although the role of Wnt signaling in endocrine pancreas development is somewhat controversial, it has been well established that Wnt signaling is required for exocrine pancreas development (3, 15, 24). Loss of Sostdc1 does not grossly affect exocrine development despite the essential need for tightly regulated Wnt activity during this time period. Although we did not detect changes in expression of Wnt target genes in islets isolated postnatally (Fig. 5), it remains possible that the Wnt pathway is altered during development in the absence of Sostdc1. Overall, it appears that deletion of Sostdc1 has a specific regulatory effect on the Bmp pathway in pancreatic islets. Despite being a dual Bmp and Wnt antagonist, the loss of Sostdc1 did not result in significant modifications in expression of the Wnt responsive genes assayed in this study. This came as a surprise as, in HEK293 cells, Sostdc1 preferentially interacts with the LRP6 receptor over binding to Bmp4 (10).

We observed divergent insulin secretion and glucose homeostasis phenotypes in animals lacking Sostdc1 and hypothesized that this discrepancy was related to genetic variability. Exploring this idea is relevant not only to the present study, but also to the human population, which is composed of individuals of mixed genetic background. Murine phenotypes observed on mixed genetic backgrounds may better mimic complex genetic traits in people. Since C57Bl/6 mice are predisposed to type 1 diabetes (19), we predicted that animals with a greater contribution of C57Bl/6 to their genetic background would exhibit improved insulin secretion and glucose homeostasis in the absence of Sostdc1. However, we were unable to correlate low FBG (<125 mg/dl) or enhanced insulin secretion with a higher percentage of C57Bl/6 alleles (19), which did not exhibit a developmental phenotype. Although we did not detect changes in expression of Wnt target genes in islets isolated postnatally (Fig. 5), it remains possible that the Wnt pathway is altered during development in the absence of Sostdc1. Overall, it appears that deletion of Sostdc1 has a specific regulatory effect on the Bmp pathway in pancreatic islets. Despite being a dual Bmp and Wnt antagonist, the loss of Sostdc1 did not result in significant modifications in expression of the Wnt responsive genes assayed in this study. This came as a surprise as, in HEK293 cells, Sostdc1 preferentially interacts with the LRP6 receptor over binding to Bmp4 (10).

Our data reveal that loss of Sostdc1 results in decreased expression of Bmp-responsive genes and Bmp and Wnt modulators in islets after HFD feeding for 8 wk (Fig. 5C). These results were surprising to us and contrary to our original hypothesis but may reflect a novel means of antagonist regulation in islets. The slight reduction of Sostdc1 and significant decrease in Ctgf expression seen in control animals on HFD for 8 wk compared with chow diet implies a mechanism in which expression of dual Bmp/Wnt modulators is downregulated in the setting of increased metabolic demand. Given that Bmp and Wnt signals positively regulate islet function, decreased expression of antagonists of either of these pathways may improve or maintain glucose homeostasis in a situation of metabolic stress. It is unclear why expression of the Bmp-selective inhibitor Gremlin trends toward an increase in expression in control animals after 8 wk of HFD feeding. Gremlin is involved in the differentiation of preadipocytes to mature adipocytes, and therefore may be systemically upregulated during exposure to HFD (7). Gremlin has also been implicated in pancreatic cancer and the pathology of diabetic nephropathy (16) but has not been studied in normal pancreas function. Of the three Bmp and Wnt modulators assessed, Gremlin is the most specific of antagonists, targeting only the Bmp pathway. This specificity may affect β-cell function in control animals on HFD; however, this needs to be tested directly. Nonetheless, we observed consistent reductions in both Ctgf and Gremlin expression in the Sostdc1 mutant background after 8 wk of HFD feeding. As described above, we also observed reductions in Smad1 and Nkx6.1 expression in Sostdc1 mutant islets and a trend toward reduced Glut2 expression. As an integral part of the Bmp signaling pathway, Smad1 mediates expression of Bmp-responsive genes, and it is therefore possible that decreased expression of Smad1 alone may contribute to the decreased levels of other Bmp-related genes. Roles for Nkx6.1 in endocrine development and differentiation have been established, but its function in the adult pancreas is less clear. In vitro studies showed that adenoviral mediated overexpression of Nkx6.1 in rat and human islets resulted in augmented insulin secretion and β-cell proliferation (16). Conversely, in vivo overexpression of Nkx6.1 in adult murine β-cells did not result in significant alterations in glucose homeostasis, insulin secretion, or β-cell proliferation (19). It has not yet been evaluated how reduced Nkx6.1 expression affects adult endocrine function. Nonetheless, Nkx6.1 is a Bmp-responsive gene in islets, and its downregulation in our Sostdc1 mutant mice suggests a reduction in Bmp pathway activity with prolonged HFD exposure.

In an effort to analyze Bmp pathway activity, we also examined whether loss of Sostdc1 would increase activity of a BRE-LacZ reporter transgene in the setting of HFD exposure. We initially hypothesized that the absence of Sostdc1 would enhance Bmp signaling activity in islets when animals were exposed to HFD, and we expected to observe β-Gal activity in the islets of Sostdc1 KO animals carrying the BRE-LacZ transgene. We did not find evidence that this reporter is active in islets of Sostdc1-null animals. This may be because the animals were not exposed to HFD for a sufficient amount of time to activate the transgene; however, we observed a significant increase in expression of the Bmp-responsive gene Glut2 after 4 wk of HFD, implying that at least some Bmp target genes are increased at this time. It may also be that the BRE-LacZ transgene is unable to be expressed in islets, although its expression was verified in the kidneys of the same animals.

Given the available published data, it is challenging to explain how decreased expression of Bmp-responsive genes is associated with improved glucose homeostasis and insulin dysfunction. This discrepancy was related to genetic variability. Exploring this idea is relevant not only to the present study, but also to the....
secretion. Recent literature suggests, however, that the deletion of \textit{Id1}, a Bmp-responsive gene, protects animals from HFD-induced obesity and enhances insulin secretion (1). This implies that modulation of specific components of the Bmp pathway may have differing effects on the $\beta$-cell. We propose that in the \textit{Sostdc1} mutant background there is a significant reduction in expression of Bmp and Wnt modulators after exposure to HFD that is not observed in control animals (Figs. 5 and 6). We hypothesize that reduced expression of Bmp antagonists enables maintenance of sufficient levels of Bmp signaling activity to maintain glucose homeostasis in the setting of increased metabolic demand. Control animals fail to reduce expression of Bmp and Wnt modulators. Bmp signaling activity is augmented in control animals to counteract the presence of these antagonists; however, impairments in insulin secretion and glucose homeostasis still occur. The changes in signaling activity are reflected in the expression levels of Bmp target genes; expression of genes such as \textit{Smad1} and \textit{Nkx6.1} are reduced in \textit{Sostdc1-null} animals compared with controls.

In conclusion, we provide evidence that loss of \textit{Sostdc1} enhances insulin secretion and glucose homeostasis in animals under metabolic stress, in part by reducing expression of other Bmp and Wnt modulators. Changes in Bmp-responsive gene expression are present in animals lacking \textit{Sostdc1}, which implies that \textit{Sostdc1} preferentially targets the Bmp pathway in the setting of the pancreatic islet. To our knowledge, this is the first study to provide evidence for diet-induced regulation of Bmp and Wnt modulators as well as coordinated regulation of the modulators themselves. These data further support a role for Bmp signaling in islet function as shown by others (5, 17) and suggest that factors that limit Bmp antagonist activity could potentiate the positive effects that Bmp has on islet function. Future studies examining the loss of \textit{Sostdc1}, \textit{CTGF}, and \textit{Gremlin} (either alone or in combination) in adult islet function and regulation of Bmp and Wnt signaling should elucidate whether these extracellular regulators may be targets for drug therapy to enhance islet function.

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DISCLOSURES

Aris N. Economides is an employee of Regeneron Pharmaceuticals.

AUTHOR CONTRIBUTIONS


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