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The role of noggin in regulation of high glucose-induced apoptosis and insulin secretion in INS-1 rat beta cells

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ABSTRACT

Objective(s): The purpose of this study was to investigate the effects of Noggin on high glucose-induced apoptosis and insulin secretion in pancreatic beta cells.
Materials and Methods: Different concentrations of glucose were used to examine their effects on INS-1 rat beta cells *in vitro*. When specific siRNA targeting Noggin and recombinant Noggin were added, apoptosis and insulin secretion were measured, respectively to determine their effects in INS-1 cells.
Results: Glucose stimulated the expression of Noggin in a dose-dependent manner. Knockdown of Noggin further increased apoptosis and reduced insulin secretion when INS-1 cells were exposed to high glucose. Conversely, administration of recombinant Noggin significantly reduced apoptotic cell number, and promoted insulin secretion. Finally, treatment with inhibitor of Smad phosphorylation exerted similar effects on cell apoptosis and insulin production to Noggin administration in glucose-treated INS-1 cells.
Conclusion: Our findings indicate that Noggin inhibits apoptosis and promotes insulin secretion in pancreatic beta cells through the inhibition of Smad signaling. Gene therapy of delivering Noggin may facilitate the treatment for patients with type 2 diabetes mellitus.

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Introduction

Type 2 diabetes mellitus (T2DM) accounts for approximately 90% of all cases of diabetes mellitus, which is one of the most common chronic diseases (1, 2). In 2010, it was estimated that about 285 million people were living with diabetes mellitus all over the world (3). Pathologically, T2DM is characterized by hyperglycemia, which may be caused by the combination of insulin resistance and inadequate insulin secretion (4, 5).

Insulin is the only known hormone that can lower blood glucose concentrations from pancreatic beta cells; in addition, insulin secretion is tightly controlled by blood glucose levels (6, 7). Thus, glucose is the primary regulator of insulin secretion (8). It is shown that glucose promoted beta cell proliferation and increased beta cell volume in 18-day fetal pancreatic explants (9). In genetically diabetic mice, upregulation of beta cell proliferation and cell volume has also been observed (10). However, as shown in studies on T2DM patients, exposure to chronic high glucose may result in a decrease in insulin synthesis and secretion of pancreatic beta cells (11). Consistently, exposure to high glucose has also been found to induce oxidative stress and apoptosis in mouse insulinoma MIN6 cells (12, 13).

Noggin is a well-known antagonist of bone morphogenetic protein (BMP) signaling. With very high (picomolar) affinities to BMPs, Noggin prevents BMPs from binding to their receptors, and inhibits the initiation of BMP signaling (14, 15). Both BMP2 and BMP4 were expressed in pancreatic islets, while administration of BMP2 and BMP4 are found to inhibit the proliferation of primary mouse beta cells (16). In addition, a single injection of BMP9 at 5 mg/kg reduced blood glucose level in normal and diabetic mice model, indicating that BMP-9 may play an important role in insulin release from pancreatic beta cells (17). However, the underlying mechanism by which BMPs regulate beta cell function remains largely unclear. Yet, the effect of Noggin on beta cells has not been determined.

It was previously shown that Smad signaling regulates islet cell proliferation and development (18, 19). In the present study, we aimed to investigate

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the role of INS-1 in apoptosis and insulin secretion in pancreatic beta cells induced by high glucose and the potential involvement of Smad signaling in INS-1's function.

Materials and Methods

Reagents

Recombinant rat Noggin protein and Lipofectamine® RNAiMAX Reagent were purchased from Life Technologies (Carlsbad, CA, USA). LDN-193189 was obtained from Sigma-Aldrich (St. Louis, MO, USA). Lipofectamine® RNAiMAX Reagent was used for siRNA transfection according to manufacturer's instructions.

Cell Culture

INS-1 cells (rat pancreatic β cell line, passages 15-30) were maintained in TPMI-1640 supplemented with 10% fetal bovine serum (FBS, Life Technologies), 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1 mM sodium pyruvate, 2 mM l-glutamine, and 50 μM 2-mercaptoethanol. INS-1 cells were cultured at 37 ˚C in a humidified atmosphere containing 5% CO_2.

Quantitative PCR (qPCR)

Total RNA from INS-1 cells was extracted using TRIzol (Life Technologies) according to manufacturer's instructions. cDNA was synthesized from 2 μg aliquots of RNA using the SuperScript II RNase H-Reverse Transcriptase kit (Life Technologies). qPCR was performed on the CFX96™ Touch Real-Time PCR Detection System using SYBR Green Supermix (Bio-Rad, Hercules, CA, USA). Comparisons were made using the $2^{-\Delta\Delta Ct}$ method, and were normalized to β-actin expression. The primer sets used were as follows: *insulin*, forward: 5'-GGCTTAACCTAAACGCCACA-3' and reverse: 5'-GGGACCGTCCAAGTTTGTAA-3'; *Noggin*, forward: 5'-TTGAATTCATGGAGCGCTGCCCCAGCCT-3', and reverse: 5'-GGGTCGACCTAGCAGGAACACTTACA-CTCGG-3'; *β-actin*, forward: 5'-TGGAATCCTGTGG-CATCC-3' and reverse: 5'-TCGTACTCCTGCTTGCTG-3'.

Western blot

Total cellular protein was extracted using radioimmunoprecipitation assay (RIPA) buffer with proteinase inhibitor (Boster, Wuhan, China). The protein concentration was measured by bicinchoninic acid (BCA) method. Protein was then separated on a 10% SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad). The membranes were blocked with 5% skimmed milk in Tris buffered saline-Tween (TBST) and incubated overnight with primary antibodies, followed by incubation with horseradish-peroxidase-conjugated antibodies. β-actin was used as loading control. The primary antibodies of Noggin, p-Smad1/5/8, Smad1 and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

ELISA assay

INS-1 cells were seeded into 24-well plates at a density of 1×10^5 cells per well. Cells were rinsed and incubated with Hanks balanced salt solution (HBSS) containing glucose (0, 5, 15 or 30 mM) for one hour at 37 ˚C. Secreted insulin was measured by enzyme-linked immunosorbent assay (ELISA) using UltraSensitive Rat Insulin ELISA Kit (Crystal Chem Inc., Downer Grove, IL, USA) according to the manufacturer's protocol.

Apoptosis assay

The percentage of apoptotic cells were assessed using fluorescein isothiocyanate (FITC) Annexin V Apoptosis Detection Kit following the manufacturer's instructions (BD Bioscience, Franklin Lakes, NJ, USA). Analysis was performed with a fluorescence-activated cell sorting (FACS) Calibur (BD Biosciences).

Statistical analysis

All experiments were performed three times. Results were presented as mean±SD. Statistical analysis was performed using one-way analysis of variance (ANOVA). $P<0.05$ was considered as significantly statistical difference. All data was analyzed by SPSS 13.0 software.

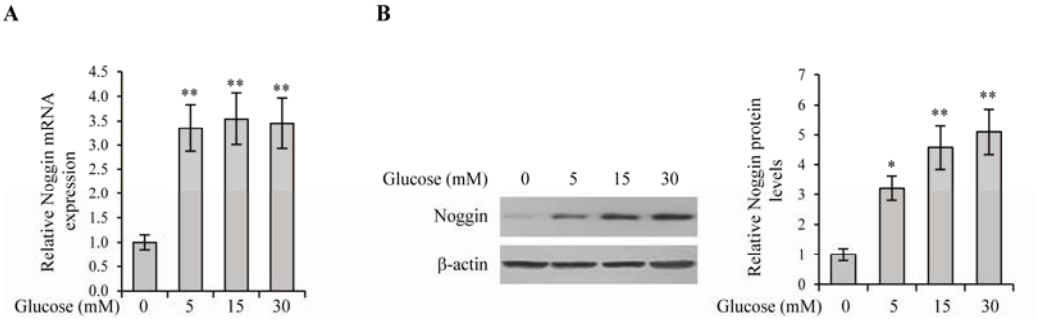

Figure 1. High glucose promoted Noggin expression in INS-1 cells. (A) RT-PCR analysis of Noggin expression in INS-1 cells treated with glucose (0, 5, 15 or 30 mM) for 3hr. (B) Western blot analysis of Noggin protein expression in INS-1 cells treated with glucose (0, 5, 15 or 30 mM) for 24 hr. Representative image was shown on left panel and quantification of bands was shown on the right panel. *P<0.05, **P< 0.01 compared to cells treated with glucose (0 mM)

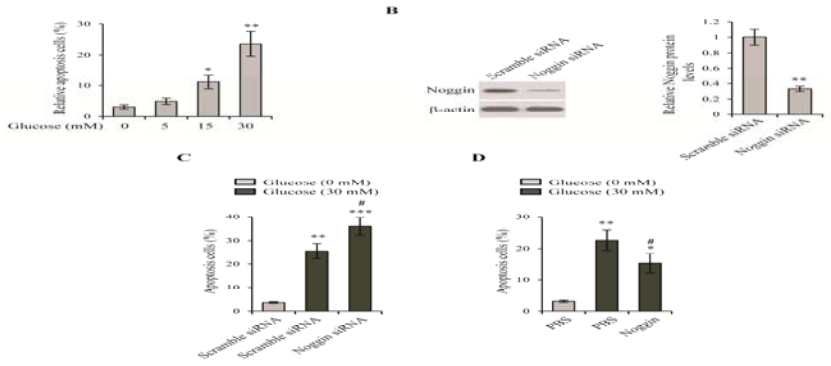

Figure 2. Noggin inhibited high glucose-induced apoptosis in INS-1 cells. (A) Apoptosis assay in INS-1 cells treated with glucose at indicated concentration for 24 hr. **P*<0.05, ***P*<0.01 compared to cells treated with glucose (0 mM). (B) Western blot analysis of Noggin protein in INS-1 cells transfected with Scramble siRNA or Noggin siRNA for 24 hr(left) and quantification of Noggin protein levels was shown (right). ***P*<0.01 compared to cells transfected with Scramble siRNA. (C) Apoptosis assay in INS-1 cells transfected with Scramble siRNA or Noggin siRNA treated with glucose (0 or 30 mM) for 24 hr. ***P*<0.01, *** *P*<0.001 compared to cells treated with glucose (0 mM). #*P*<0.05 compared to cells transfected with Scramble siRNA. (D) Apoptosis assay in INS-1 cells treated with glucose (0 or 30 mM) for 24 hr in the absence and presence of Noggin (200 ng/ml). **P*<0.05, ***P*<0.01 compared to cells treated with glucose (0 mM). #*P*<0.05 compared to PBS + Glucose (30 mM) group

Results

High glucose promoted Noggin expression in INS-1 cells

We first assessed the expression of Noggin in rat β cell line INS-1 cells in response to glucose stimulation. INS-1 cells were treated with glucose (0, 5, 15 or 30 mM) for 3 hr, and then Noggin mRNA expression was examined by RT-PCR. As shown in Figure 1A, Noggin expression was significantly elevated by glucose stimulation. We further performed western blot to evaluate the protein level of Noggin in INS-1 cells treated with glucose for 24 hr. The results confirmed that Noggin expression was induced by high glucose (Figure 1B).

Noggin inhibited high glucose-induced apoptosis in INS-1 cells

High glucose has been shown to be toxic to β-cells. We found that glucose induced apoptosis of INS-1 cells in a dose-dependent manner (Figure 2A). To explore the role of Noggin in high glucose-induced apoptosis of β-cells, Noggin expression was knocked down (Figure 2B). As shown in Figure 2C, siRNA-mediated depletion of Noggin further enhanced glucose-induced apoptosis of INS-1 cells. Conversely, administration of exogenous Noggin partly rescued cells from apoptosis induced by glucose (Figure 2D).

Noggin is required for glucose-induced insulin secretion in INS-1 cells

We sought to examine whether Noggin is involved in insulin secretion of β cells. INS-1 cells were treated with glucose (0, 5, 15 or 30 mM) for 3 hr, and insulin mRNA expression was assessed by RT-PCR. As expected, treatment with 15 mM glucose resulted in the highest insulin secretion in INS-1 cells (Figure 3A, 3B). Furthermore, depletion of Noggin in INS-1 cells inhibited insulin secretion stimulated by high concentration of glucose (Figure 3C, 3D). Interestingly, treatment INS-1 cells with Noggin protein markedly increased the insulin secretion (Figure 3E and F).

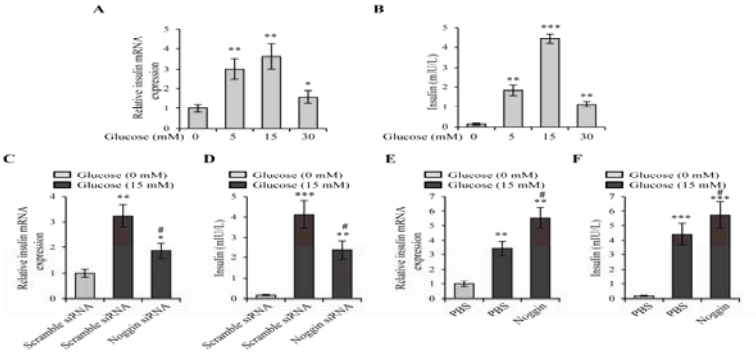

Figure 3. Noggin is required for glucose-induced insulin secretion in INS-1cells. (A) RT-PCR analysis of insulin expression in INS-1 cells treated with various doses of glucose for 3 hr. (B) ELISA analysis of insulin secretion in INS-1 cells treated with various doses of glucose for 3 hr. (C-D) Insulin mRNA (C) and protein (D) expression in INS-1 cells transfected with Scramble siRNA or Noggin siRNA treated with glucose (0 or 15 mM). #*P*<0.05 compared to Scramble siRNA + glucose (15 mM) group. (E-F) Insulin mRNA (E) and protein (F) expression in INS-1 cells treated with glucose (0 or 15 mM) in the absence and presence of Noggin (200 ng/ml). #*P*<0.05 compared to PBS + glucose (15 mM) group. **P*<0.05, ***P*<0.01, *** *P*<0.001 compared to cells treated with glucose (0 mM)

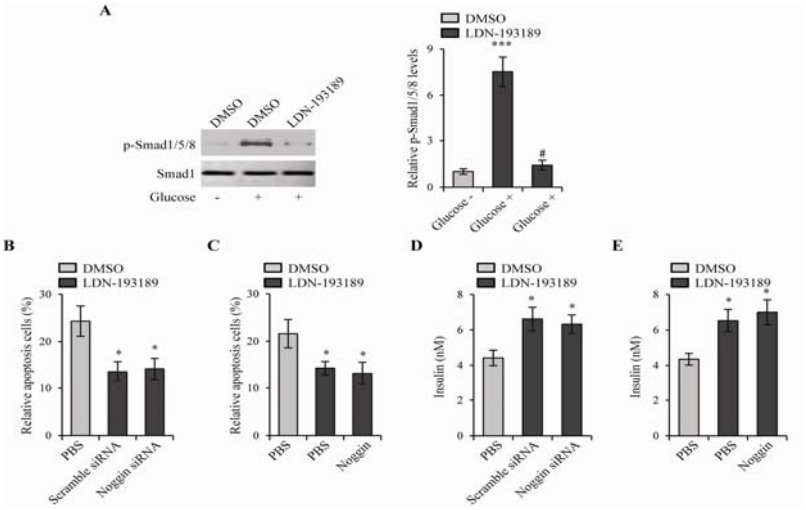

Figure 4. Noggin regulated INS-1 cell apoptosis and function via Smad signaling. (A) Western blot analysis of p-Smad1/5/8 in INS-1 cells treated with glucose (30 ng/ml) for 30 min in the absence or presence of LDN-193189 (100 nM). Quantification of western blot was shown (right). ***P<0.001 compared to cells treated with glucose (0 mM). #P<0.05 compared to LDN-193189 + glucose group. (B) Apoptosis assay in INS-1 cells transfected with Scramble siRNA or Noggin siRNA treated with glucose (30 mM) for 24 hr in the absence or presence of LDN-193189 (100 nM). (C) Apoptosis assay in INS-1 cells (PBS + DMSO, Scramble siRNA + LDN-193189, Noggin siRNA + LDN-193189) treated with glucose (30 mM). (D) RT-PCR analysis of insulin levels in INS-1 cells transfected with Scramble siRNA or Noggin siRNA treated with glucose (30 mM) for one hour in the absence or presence of LDN-193189 (100 nM). (E) ELISA analysis of insulin levels in INS-1 cells (PBS + DMSO, Scramble siRNA + LDN-193189, Noggin siRNA + LDN-193189) treated with glucose (15 mM). *P<0.05 compared to cells (PBS + DMSO)

Noggin regulated INS-1 cell apoptosis and insulin secretion via Smad signaling

Since Noggin is a bone morphogenetic protein (BMP) antagonist (20), we investigated whether Noggin exerted its effects in glucose-stimulated cells through inhibition of Smad signaling. As shown in Figure 4A, glucose (30 mM) induced phosphorylation of Smad1/5/8, which was suppressed by addition of LDN-193189 (Figure 4A). Then, we performed cell apoptosis and ELISA assays. As shown in Figure 4B-4E, Noggin failed to further suppress cell apoptosis and insulin production in glucose-stimulated INS-1 cells, when Smad signaling was blocked by LDN-193189 treatment.

Discussion

In the present study, we first determined whether high glucose induces Noggin expression. Further, we investigated the role of Noggin in regulation of apoptosis and insulin secretion in INS-1 cells. Using a specific inhibitor of Smad phosphorylation, we found that Noggin exerts its effects through inhibition of Smad signaling.

The tight association between high glucose and pancreatic beta cell function has been long implicated. Under physiological conditions, glucose intake stimulates beta cells to synthesize and secret insulin (8, 21). But chronic hyperglycemia, such as high blood glucose levels in patients with T2DM, is toxic to beta cells, leading to beta cell apoptosis (22).

In our study, different concentrations of glucose were used to examine their effects on INS-1 cells *in vitro*. While glucose induced apoptosis of INS-1 cells in a dose-dependent manner, 15 mM glucose was the most effective concentration for insulin secretion of INS-1 cells, as determined by RT-PCR and ELISA assay. Accordingly, our finding showed the toxicity of glucose on pancreatic beta cells. Glucose concentration is essential for cellular function of pancreatic beta cells. Furthermore, Martina *et al* reported that exposure to high glucose concentration led to a decrease in tricarboxylic acid cycle metabolism, and an alteration in fatty acid composition (23), indicating that high glucose affects the central metabolism in pancreatic beta cells (24).

The development of T2DM is accompanied by progressive decrease in beta cell function, which is largely caused by beta cell apoptosis (25, 26). Smad proteins were found to mediate apoptosis in many cell types and tissues (27). In regard to pancreatic cells, the role of Smad in regulation of apoptosis has not been reported so far. In this study, we found that inhibition of Smad1/5/7 phosphorylation by LDN-193189 significantly suppressed high glucose-induced apoptosis in INS-1 cells, indicating that Smad may play an essential role in the process of programmed cell death. Furthermore, LDN-193189 treatment mimics the inhibitory effects of Noggin on the cell apoptosis in glucose-treated INS-1 cells. Since Noggin is an antagonist of BMP signaling, these results suggest that high glucose may induce apoptosis of INS-1 cells through Smad-mediated BMP signaling.

BMP signaling is also associated with insulin production and secretion in pancreatic beta cells (28,

29). In response to high glucose, both phosphorylation levels of Smad1/5/8 and expression of Noggin were elevated. In addition, we found that knockdown of Noggin reduced high glucose-stimulated insulin secretion, and administration of recombinant Noggin enhanced high glucose-stimulated insulin secretion. Additionally, treatment with LDN-193189, a small molecule BMP inhibitor, increased insulin secretion in high glucose-stimulated INS-1 cells. Our results suggest that activation of BMP signaling by high glucose repressed insulin secretion from pancreatic beta cells. In contrast to our findings, it is reported that deletion of bone morphogenetic protein receptor type 1 A (BMPR1A) in mouse beta cells led to reduced expression of genes associated with insulin production, and these mice showed signs of glucose intolerance within 3 months of life (30). It should be noted that the concentration of glucose is slightly higher than the blood glucose levels *in vivo* (31). We also suggest that this discrepancy may arise from differences in the effects of BMPs on proliferation and crosstalk between BMP signaling and other pathways (16, 32). On the other hand, BMP signaling is involved in the generation of pancreatic progenitors form human embryonic stem cells (33, 34). But, the role of BMP signaling in regulation of differentiation from pancreatic progenitors to beta cells still needs further investigation.

Conclusion

In summary, the results of this study showed that high glucose stimulated Noggin expression in INS-1 cells. siRNA-mediated depletion of Noggin increased high glucose-induced apoptosis, and reduced high glucose-induced insulin secretion. Conversely, exogenous treatment with Noggin suppressed high glucose-induced apoptosis, and enhanced high glucose-induced insulin secretion. Hence, as an extracellular factor, Noggin may provide the therapeutic potential for T2DM patients.

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References

1. Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus—present and future perspectives. Nat Rev Endocrinol 2011; 8:228-236.
2. Pinhas-Hamiel O, Zeitler P. The global spread of type 2 diabetes mellitus in children and adolescents. J Pediatr 2005; 146:693-700.
3. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010; 87:4-14.
4. Buchanan TA. Pancreatic B-cell defects in gestational diabetes: implications for the pathogenesis and prevention of type 2 diabetes. J Clin Endocrinol Metab 2001; 86:989-993.
5. Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, *et al*. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care 2009; 32:193-203.
6. Aronoff SL, Berkowitz K, Shreiner B, Want L. Glucose metabolism and regulation: beyond insulin and glucagon. Diabetes Spectr 2004; 17:183-1890.
7. Van den Berghe G. How does blood glucose control with insulin save lives in intensive care? J Clin Invest 2004; 114:1187.
8. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001; 414:799-806.
9. Kaung HC. Effect of glucose on beta cell proliferation and population size in organ culture of foetal and neonatal rat pancreases. J Embryol Exp Morphol 1983; 75:303-312.
10. Like A, Chick W. Studies in the diabetic mutant mouse: I. Light microscopy and radioautography of pancreatic islets. Diabetologia 1970; 6:207-215.
11. Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H. Glucose toxicity in β-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. Diabetes 2003; 52:581-587.
12. Zhang Z, Liew CW, Handy DE, Zhang Y, Leopold JA, Hu J, *et al*. High glucose inhibits glucose-6-phosphate dehydrogenase, leading to increased oxidative stress and β-cell apoptosis. FASEB J 2010; 24:1497-1505.
13. Kim W-H, Lee JW, Suh YH, Hong SH, Choi JS, Lim JH, *et al.* Exposure to chronic high glucose induces β-Cell apoptosis through decreased interaction of glucokinase with mitochondria downregulation of glucokinase in pancreatic β-Cells. Diabetes 2005; 54:2602-2611.
14. McMahon JA, Takada S, Zimmerman LB, Fan C-M, Harland RM, McMahon AP. Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite. Genes Dev 1998; 12:1438-1452.
15. Canalis E, Economides AN, Gazzerro E. Bone morphogenetic proteins, their antagonists, and the skeleton. Endocr Rev 2003; 24:218-235.
16. Bruun C, Christensen GL, Jacobsen ML, Kanstrup MB, Jensen PR, Fjordvang H, *et al*. Inhibition of beta cell growth and function by bone morphogenetic proteins. Diabetologia 2014; 57:2546-2554.
17. Chen C, Grzegorzewski KJ, Barash S, Zhao Q, Schneider H, Wang Q, *et al*. An integrated functional genomics screening program reveals a role for BMP-9 in glucose homeostasis. Nat Biotechnol 2003; 21:294-301.
18. El-Gohary Y, Tulachan S, Wiersch J, Guo P, Welsh C, Prasadan K, *et al.* A smad signaling network regulates islet cell proliferation. Diabetes 2014; 63:224-236.
19. El-Gohary Y, Tulachan S, Guo P, Welsh C, Wiersch J, Prasadan K, *et al.* Smad signaling pathways regulate

pancreatic endocrine development. Dev Biol 2013; 378:83-93.
20. He F, Xiong W, Wang Y, Matsui M, Yu X, Chai Y, *et al*. Modulation of BMP signaling by Noggin is required for the maintenance of palatal epithelial integrity during palatogenesis. Dev Biol 2010; 347:109-121.
21. Moynihan KA, Grimm AA, Plueger MM, Bernal-Mizrachi E, Ford E, Cras-Méneur C, *et al*. Increased dosage of mammalian Sir2 in pancreatic β cells enhances glucose-stimulated insulin secretion in mice. Cell Metab 2005; 2:105-117.
22. Robertson RP, Harmon J, Tran POT, Poitout V. β-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. Diabetes 2004; 53:S119-S124.
23. Wallace M, Whelan H, Brennan L. Metabolomic analysis of pancreatic beta cells following exposure to high glucose. Biochim Biophys Acta 2013; 1830:2583-2590.
24. Matschinsky FM. Glucokinase as glucose sensor and metabolic signal generator in pancreatic β-cells and hepatocytes. Diabetes 1990; 39:647-652.
25. Donath MY, Ehses JA, Maedler K, Schumann DM, Ellingsgaard H, Eppler E, *et al*. Mechanisms of beta-Cell Death in Type 2 Diabetes. Diabetes. 2005; 54:S108-113.
26. Leonardi O, Mints G, Hussain MA. Beta-cell apoptosis in the pathogenesis of human type 2 diabetes mellitus. Eur J Endocrinol 2003; 149:99-102.
27. Schuster N, Krieglstein K. Mechanisms of TGF-β-mediated apoptosis. Cell Tissue Res 2002; 307:1-14.
28. Smart NG, Apelqvist ÅA, Gu X, Harmon EB, Topper JN, MacDonald RJ, *et al*. Conditional expression of Smad7 in pancreatic β cells disrupts TGF-β signaling and induces reversible diabetes mellitus. PLoS Biol 2006; 4:e39.
29. Gannon M. BuMP-ing up insulin secretion by pancreatic β cells. Cell Metab 2007; 5:157-159.
30. Goulley J, Dahl U, Baeza N, Mishina Y, Edlund H. BMP4-BMPR1A signaling in β cells is required for and augments glucose-stimulated insulin secretion. Cell Metab 2007; 5:207-219.
31. Alberti K, Zimmet Pf. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998; 4:539-553.
32. Heldin C-H, Miyazono K, Ten Dijke P. TGF-β signalling from cell membrane to nucleus through SMAD proteins. Nature 1997; 390:465-471.
33. Xu X, Browning VL, Odorico JS. Activin, BMP and FGF pathways cooperate to promote endoderm and pancreatic lineage cell differentiation from human embryonic stem cells. Mech Dev 2011; 128:412-427.
34. Mfopou JK, Chen B, Mateizel I, Sermon K, Bouwens L. Noggin, retinoids, and fibroblast growth factor regulate hepatic or pancreatic fate of human embryonic stem cells. Gastroenterology 2010; 138:2233-2245.e14.