Methods and reagents for treating diabetes

Cross-Reference

This application claims priority to U.S. Provisional Patent Application Serial No. 62/700583 filed July 19, 2018 and U.S. Provisional Patent Application Serial No. 62/700587 filed July 19, 2018, each incorporated by reference in its entirety.

Background

The International Diabetes Federation (IDF) has estimated that the number of people with diabetes in 2011 was 366 million and this number is expected to grow to 550 million by 2030. The majority of patients are suffering from type-2 diabetes mellitus (T2DM). There is a link between obesity, insulin resistance and the development of T2DM, but the precise underlying mechanisms remain unknown and it becomes paramount to identify potential therapeutic targets that can ultimately address this crucial problem.

Classical target tissues subjective to insulin resistance in T2DM are muscle, liver and fat. These peripheral tissues maintain glucose homeostasis by effectively responding to insulin and failure of insulin to activate its receptors and the downstream signaling cascades result in defect glucose handling. In addition, insulin can stimulate its receptors on the pancreatic β-cell and thereby directly contribute to a positive feedback loop for insulin biosynthesis and secretion.

Summary

In one example, the disclosure provides methods for treating or limiting development of diabetes, comprising transplanting into the eye of a subject with diabetes or at risk of diabetes an amount effective to treat or limit development of diabetes of insulin-producing cells engineered to reduce expression of a β3 subunit of Cav (Cavβ3). In one example, the insulin-producing cells are engineered to disrupt each copy of the Cavβ3 gene. In another example, the insulin-producing cells are engineered to reduce expression of apoCIII, such

as by disrupting each copy of the apoCIII gene. In another example, the insulin-producing cells are engineered to reduce expression of one or more chemokines, including but not limited to CCL2, CCL3, CCL5, CXCL1, CXCL9, CXCL10, and/or CXCL11, and/or one or more cytokines including but not limited to IL-6 and/or IL-8. In one example, the insulin-producing cells are engineered to reduce expression of one or more major histocompatibility complex (MHC) class I proteins, including but not limited to human HLA-A, HLA-B, and/or HLA-C genes. In a further example, the insulin-producing cells are engineered to disrupt each copy of the one or more MHC class I proteins. In one example, the insulin-producing cells are engineered to increase expression of one or more proteins beneficial to insulin producing cells, including but not limited to GLP1 receptors, insulin receptors, and/or cytokine IL1B. In another example, the insulin-producing cells comprise isolated pancreatic islets or isolated pancreatic β cells, such as isolated human pancreatic islets or isolated human pancreatic β cells. In a further example, the transplantation into the eye involves transplantation into the anterior chamber of the eye, including but not limited to transplantation into the anterior chamber of the eye involves injection through the cornea. In one example, the subject, such as a human subject, has diabetes and the transplanting comprising transplanting into the eye of a subject with diabetes (including but not limited to type 1 and type 2 diabetes) an amount effective to treat diabetes. In another example, the subject, such as a human subject, is at risk of diabetes and the transplanting comprising transplanting into the eye of a subject at risk of diabetes (including but not limited to a risk of type 1 and type 2 diabetes) an amount effective to limit development of diabetes. In a further example, the subject, such as a human subject, overexpresses Cavβ3 compared to a control.

In one aspect, the present disclosure refers to use of insulin-producing cells engineered to reduce expression of a β3 subunit of Cav (Cavβ3) in the manufacture of a medicament for treating or limiting development of diabetes, wherein the medicament is to be transplanted into the eye of a subject with diabetes or at risk of diabetes.

In another example, the disclosure provides recombinant cells comprising an insulin-producing cell engineered to reduce expression of a β3 subunit of Cav (Cavβ3). In one example, the insulin-producing cells are engineered to disrupt each copy of the Cavβ3 gene. In another example, the insulin-producing cells are engineered to reduce expression of apoCIII, such as by disrupting each copy of the apoCIII gene. In another example, the insulin-producing cells are engineered to reduce expression of one or more chemokines, including but not limited to CCL2, CCL3, CCL5, CXCL1, CXCL9, CXCL10, and/or CXCL11, and/or one or more cytokines including but not limited to IL-6 and/or IL-8. In one example, the insulin-producing cells are engineered to reduce expression of one or more major histocompatibility complex (MHC) class I proteins, including but not limited to human

HLA-A, HLA-B, and/or HLA-C genes. In a further example, the insulin-producing cells are engineered to disrupt each copy of the one or more MHC class I proteins. In one example, the insulin-producing cells are engineered to increase expression of one or more proteins beneficial to insulin producing cells, including but not limited to GLP1 receptors, insulin receptors, and/or cytokine IL1B. In another example, the insulin-producing cells comprise isolated pancreatic islets or isolated pancreatic β cells, such as isolated human pancreatic islets or isolated human pancreatic β cells.

In another aspect, the present disclosure refers to a recombinant cell comprising an insulin-producing cell engineered to reduce expression of a β3 subunit of Cav (Cavβ3).

Description of the Figures

Figure 1. $Ca_v\beta_3$ expression and $[Ca^{2+}]_i$ dynamics were altered in islets from ob/ob mice. (**A**) Left panel shows protein levels of $Ca_v\beta_3$ in islets from control lean mice and ob/ob mice. Right panel shows relative quantification of $Ca_v\beta_3$ protein expression in left panel (n = 5, 40 islets in each case). (**B,C**) Effects of 11 mM glucose on $[Ca^{2+}]_i$ in islets from control and ob/ob mice. Representative traces out of 39 for lean and ob/ob islets are shown. (**D**) First peak ratios of glucose-induced $[Ca^{2+}]_i$ changes in islets from control lean and ob/ob mice. (**E**) Oscillation periods of glucose-induced $[Ca^{2+}]_i$ changes in islets from control lean and ob/ob mice. (**F**) Oscillation amplitudes of glucose-induced $[Ca^{2+}]_i$ changes in islets from control lean mice and ob/ob mice. (**G**) Effects of 25 mM KCl on $[Ca^{2+}]_i$ in dissociated islet cells from control lean (black) and ob/ob (red) mice. Representative traces in dissociated islet cells from control lean and ob/ob mice are shown. (**H**) Peak ratios of $[Ca^{2+}]_i$ changes induced by 25 mM KCl in dissociated islet cells from lean (black) and ob/ob (red) mice. (n = 10, each experiment included 50 single cells). (**I**) Glucose-induced insulin release in islets from control lean and ob/ob mice. The islets were treated with 3 mM or 11 mM glucose for 30 min (n = 5, 10 islets in each case). (**J**) Left panel shows intraperitoneal glucose tolerance test in control lean and ob/ob mice (n = 5 each). Right panel shows comparison of areas under the curves from left panel. Data are presented as the mean ± SEM, $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

Figure 2. $Ca_v\beta_3$ expression and $[Ca^{2+}]_i$ dynamics were altered in islets of HFD mice. (**A**) Left panel shows protein levels of $Ca_v\beta_3$ in islets from NCD and HFD B6 mice. Right panel shows relative quantification of $Ca_v\beta_3$ protein expression in left panel (n = 5, 40 islets in each case). (**B,C**) Effects of 11 mM glucose on $[Ca^{2+}]_i$ in islets from NCD and HFD B6 mice. Representative traces out of 30 for NCD and HFD islets are shown. (**D**) First peak ratios of glucose-induced $[Ca^{2+}]_i$ changes in islets from NCD and HFD B6 mice. (**E**) Oscillation periods of glucose-induced $[Ca^{2+}]_i$ changes in islets from NCD and HFD B6 mice. (**F**)

Claims

1. Use of insulin-producing cells engineered to reduce expression of a β3 subunit of Cav (Cavβ3) in the manufacture of a medicament for treating or limiting development of diabetes, wherein the medicament is to be transplanted into the eye of a subject with diabetes or at risk of diabetes.

2. The use of claim 1, wherein the insulin-producing cells are engineered to disrupt each copy of the Cavβ3 gene.

3. The use of claim 1 or 2, wherein the insulin-producing cells are engineered to reduce expression of apoCIII.

4. The use of claim 3, wherein the insulin-producing cells are engineered to disrupt each copy of the apoCIII gene.

5. The use of any one of claims 1-4, wherein the insulin-producing cells are engineered to reduce expression of one or more chemokines, including but not limited to CCL2, CCL3, CCL5, CXCL1, CXCL9, CXCL10, and/or CXCL11, and/or one or more cytokines including but not limited to IL-6 and/or IL-8.

6. The use of claim 5, wherein the insulin-producing cells are engineered to disrupt each copy of the one or more chemokine genes and/or the one or more cytokine genes.

7. The use of any one of claims 1-6, wherein the insulin-producing cells are engineered to reduce expression of one or more major histocompatibility complex (MHC) class I proteins.

8. The use of claim 7, wherein the insulin-producing cells are engineered to disrupt each copy of the one or more MHC class I proteins.

9. The use of any one of claims 1-8, wherein the insulin-producing cells are engineered to increase expression of one or more proteins beneficial to insulin producing cells, including but not limited to GLP1 receptors, insulin receptors, and/or cytokine IL1B.

10. The use of any one of claims 1-9, wherein the insulin-producing cells comprise isolated pancreatic islets or isolated pancreatic β cells, such as isolated human pancreatic

islets or isolated human pancreatic β cells.

11. The use of any one of claims 1-10, wherein the transplantation into the eye involves transplantation into the anterior chamber of the eye.

12. The use of claim 11, wherein the transplantation into the anterior chamber of the eye involves injection through the cornea.

13. The use of any one of claims 1-12, wherein the subject, such as a human subject, has diabetes and the transplantation comprises transplantation of the medicament into the eye of a subject with diabetes an amount effective to treat diabetes.

14. The use of claim 14, wherein the subject has type 2 diabetes.

15. The use of claim 14, wherein the subject has type 1 diabetes.

16. The use of any one of claims 1-12, wherein the subject, such as a human subject, is at risk of diabetes and the transplantation comprises transplantation of the medicament into the eye of a subject at risk of diabetes an amount effective to limit development of diabetes.

17. The use of claim 16, wherein the subject is at risk of type 2 diabetes.

18. The use of claim 16, wherein the subject is at risk of type 1 diabetes.

19. The use of any one of claims 1-18, wherein the subject, such as a human subject, overexpresses Cavβ3 compared to a control.

20. A recombinant cell comprising an insulin-producing cell engineered to reduce expression of a β3 subunit of Cav (Cavβ3).

21. The recombinant cell of claim 20, wherein the insulin-producing cell is engineered to disrupt each copy of the Cavβ3 gene.

22. The recombinant cell of claim 20 or 21, wherein the insulin-producing cell is engineered to reduce expression of apoCIII.

23. The recombinant cell of claim 22, wherein the insulin-producing cell is engineered to disrupt each copy of the apoCIII gene.

24. The recombinant cell of any one of claims 20-23, wherein the insulin-producing cell is engineered to reduce expression of one or more chemokines, including but not limited to CCL2/3/5, CXCL1/9/10/11, and IL-6/8.

25. The recombinant cell of claim 24, wherein the insulin-producing cell is engineered to

disrupt each copy of the one or more chemokine genes.

26. The recombinant cell of any one of claims 20-25, wherein the insulin-producing cell is engineered to reduce expression of one or more major histocompatibility complex MHC class I proteins.

27. The recombinant cell of claim 26, wherein the insulin-producing cell is engineered to disrupt each copy of the one or more MHC class I proteins.

28. The recombinant cell of any one of claims 20-27, wherein the insulin-producing cells are engineered to increase expression of one or more proteins beneficial to insulin producing cells, including but not limited to GLP1 receptors, insulin receptors, and/or cytokine IL1B.

29. The recombinant cell of any one of claims 20-28, wherein the insulin-producing cells comprise isolated pancreatic islets or isolated pancreatic β cells, such as isolated human pancreatic islets or isolated human pancreatic β cells.