

determination (Chassot, A. A. et al. Hum Mol Genet 17, 1264-1277, doi:10.1093/hmg/ddn016 (2008)) and they have been reported as central regulators of the canonical WNT signaling pathway (also known as WNT/ β -catenin or cWNT pathway) (Jin, Y. R. & Yoon, J. K. The R-spondin family of proteins: emerging regulators of WNT signaling. Int J Biochem Cell Biol 44, 2278-2287, doi:10.1016/j.biocel.2012.09.006 (2012)).

Despite the great deal of interest raised by the possible involvement of the cWNT pathway in pancreas maturation and function (Scheibner et al 2019, Curr Opin Cell Biol. 61:48-55), the roles and the contribution of R-spondin proteins have been poorly investigated in this organ.

In vitro analyses reported that, in the presence of Rspo1, β -cell proliferation and function are increased in the Min6 tumor-derived cell line (Wong, V. S., Yeung, A., Schultz, W. & Brubaker, P. L. R-spondin-1 is a novel beta-cell growth factor and insulin secretagogue. J Biol Chem 285, 21292-21302, doi:10.1074/jbc.M110.129874 (2010)). However, further more recent studies from the same group reported contradictory statements : Rspo1 deficiency in mice is associated with increased β -cell mass and enhanced glycemic controls (Wong, V. S., Oh, A. H., Chassot, A. A., Chaboissier, M. C. & Brubaker, P. L. Diabetologia 54, 1726-1734, doi:10.1007/s00125-011-2136-2 (2011) and Chahal et al 201, Pancreas Vol 43(1) pp 93-102).

In contrast to the latter studies, the inventors have now surprisingly shown that treatments with recombinant Rspo1 protein induce in vivo proliferation of functional pancreatic beta cells, and improve glucose tolerance and increase glucose-stimulated insulin secretion (GSIS) in mice models of diabete. In addition, they found out that upon near complete beta-cell ablation, the remaining beta-cells could be induced with Rspo1 protein administration to proliferate and reconstitute a functional beta-cells mass able to maintain euglycemia. Lastly, they showed that Rspo1 can also induce human beta-cell proliferation opening new unexpected avenues for the treatment and prevention of diabetes in human.

SUMMARY

The present disclosure refers to isolated Rspo1 proteins and their use as a medicament, preferably in the treatment of diabetes in a subject in need thereof. In one aspect, the present disclosure refers to the use of an isolated Rspo1 protein in the manufacture of a medicament for treating diabetes in a subject in need thereof.

In specific examples, said Rspo1 protein of the disclosure, is either

- (i) a protein comprising a Rspodin-1 polypeptide,
- (ii) a protein comprising a functional fragment of Rspodin-1 polypeptide, or
- (iii) a protein comprising a functional variant of Rspodin-1 polypeptide.

In specific examples, said Rspo1 protein of the disclosure is either

- 5 (i) a protein comprising a human Rspodin1 polypeptide of any one of SEQ ID NOs:2-4,
- (ii) a protein comprising a functional fragment of human Rspodin-1 polypeptide of any one of SEQ ID NOs:2-4, or
- 10 (iii) a protein comprising a functional variant of Rspodin-1 polypeptide of any one of SEQ ID NOs:2-4.

In specific examples, said Rspo1 protein of the disclosure is a protein comprising a functional fragment of Rspodin-1 polypeptide, said functional fragment preferably comprising or consisting of a polypeptide having at least 40-100 consecutive amino acid residues in the FU1 and/or FU2 domains of Rspodin1 protein, typically at least 40-100 consecutive amino acid residues of any of the polypeptides of SEQ ID NO:1-4 and SEQ ID NO:8-24.

In specific examples, said Rspo1 protein of the disclosure is a recombinant protein comprising either

- (i) any one of SEQ ID NO: 1-4 and SEQ ID NO:8-24, or
- 20 (ii) a combination of fragments of Rspo1 protein of SEQ ID NO:1, typically including the functional domain FU1 and the functional domain FU2, and, optionally the functional domain TSP.

In specific examples, said Rspo1 protein of the disclosure binds to LGR4 receptor.

In specific examples, said Rspo1 protein of the disclosure induces the proliferation of functional beta cells as determined in an in vitro beta cell proliferation assay and/or in an in vivo beta cell proliferation assay.

In specific examples, said Rspo1 protein of the disclosure is a protein comprising a functional fragment or functional variant of a native Rspodin-1 polypeptide preferably, of human R-spondin-1 of SEQ ID NO :3 or 4, and said Rspo1 protein exhibits at least 50%, 60%, 70%, 80%, 90% 100% or more of one or more of the following activities relative to said native R-spondin 1:

- (i) Binding affinity to LGR4 receptor, for example as determined by SPR assay;

- (ii) Induction of the proliferation of functional beta cells, for example as determined in an in vitro beta cell proliferation assay;
- (iii) Induction of the proliferation of functional beta cells, for example as determined in an in vivo beta cell proliferation assay;
- 5 (iv) Increase of glucose-stimulated insulin secretion (GSIS), for example as determined in an in vitro beta cell proliferation assay; or,
- (v) Increase of glucose-stimulated insulin secretion (GSIS), for example as determined in an in vivo beta cell proliferation assay.

The above functional assays are for example described in more details in the Examples
10 below.

In specific examples, said Rspo1 protein of the disclosure is a protein comprising a functional variant of R-spondin 1, wherein said functional variant comprises or essentially consists of a polypeptide having at least 70%, 80%, 90% or at least 95% identity to a native R-spondin 1 polypeptide sequence, preferably at least 70%, 80%, 90% or at least 95% identity to one of
15 polypeptides of SEQ ID NOs :1-4 and SEQ ID NO :8-24.

In specific examples, said functional variant of R-spondin 1 differs from the corresponding native R-spondin 1 sequence through only amino acid substitutions.

In specific examples, said Rspo1 protein of the disclosure is a fusion protein, for example a fusion protein comprising an Fc region of an antibody.

20 In specific examples, said Rspo1 protein of the disclosure is a pegylated or PASylated protein.

According to the present disclosure, said Rspo1 proteins are particularly useful in the treatment of diabete type 1 or type 2 and/or in inducing in vivo the proliferation of béta cells and the increase of mass of islets of Langerhans. In specific examples, a therapeutically
25 efficient amount of Rspo1 protein is administered via the subcutaneous or intravenous route to a subject in need thereof.

In specific examples of such in vivo use of the Rspo1 proteins, said subject is a human subject.

The disclosure also refers to a pharmaceutical composition comprising the Rspo1 protein as
30 defined above, and one or more pharmaceutically acceptable excipients. In one aspect, the present disclosure refers to use of a pharmaceutical composition comprising an isolated Rspo1 protein and one or more pharmaceutically acceptable excipients in the manufacture of a medicament for treating diabetes in a subject in need thereof.

In specific examples, said pharmaceutical composition further comprises one or more
35 additional pharmaceutical ingredients for treating or preventing diabetes, typically,

selected from the group consisting of cytokines, anti-viral, anti-inflammatory agents, anti-diabetic or hypoglycemic agents, cell therapy product (e.g. beta cell composition) and immune modulators.

The disclosure also refers to the use of a Rspo1 protein or an analogue as defined herein,
 5 in an in vitro method for inducing the proliferation of beta cells, typically human beta cells.

Typically, said in vitro method comprises the following:

- (i) providing induced pluripotent stem cells (iPSCs), preferably iPSCs from human cells,
- (ii) in vitro differentiating said iPSCs to β -cells of islets of Langerhans, and
- 10 (iii) culturing said differentiated β -cells under proliferating conditions,
- (iv) wherein a sufficient amount of said Rspo1 protein or analogue is added at step (ii) and/or (iii) for differentiating iPS cells and/or inducing the proliferation of said β -cells.

DETAILED DESCRIPTION

15 Definitions

In order that the present disclosure may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description.

The term "amino acid" refers to naturally occurring and unnatural amino acids (also referred to herein as "non-naturally occurring amino acids"), e.g., amino acid analogues
 20 and amino acid mimetics that function similarly to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine. Amino acid analogues refer to compounds that have the same basic chemical structure as a naturally occurring amino acid, e.g., an alpha carbon that is
 25 bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogues can have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refer to chemical compounds that have a structure that is different from
 30 the general chemical structure of an amino acid, but that function similarly to a naturally occurring amino acid. The terms "amino acid" and "amino acid residue" are used interchangeably throughout.

CLAIMS

1. Use of an isolated Rspo1 protein in the manufacture of a medicament for treating diabetes in a subject in need thereof.
- 5 2. The use of claim 1, wherein the isolated Rspo1 protein is a human R-spondin 1 polypeptide.
3. The use of claim 1 or 2, wherein the isolated Rspo1 protein comprises an amino acid
10 sequence having at least 90% identity with SEQ ID NO:3.
4. The use of any of the preceding claims, wherein the isolated Rspo1 protein binds to LGR4 receptor.
- 15 5. The use of any one of the preceding claims, wherein the isolated Rspo1 protein is a fusion protein.
6. The use of any one of the preceding claims, wherein the isolated Rspo1 protein is a fusion protein comprising an Fc region of an antibody.
- 20 7. The use of claim 6, wherein said Fc region of an antibody is fused to the human Rspo1 protein via a peptide linker.
8. The use of any of the preceding Claims, wherein the isolated Rspo1 protein is a
25 pegylated or PASylated protein.
9. The use of any of the preceding claims, wherein the diabetes is type 1 or type 2.
10. The use of any of the preceding Claims, wherein a therapeutically efficient amount of
30 Rspo1 protein is to be administered via the subcutaneous or intravenous route to the subject.
11. The use of any of the preceding Claims, wherein said subject is a human subject.
- 35 12. Use of a pharmaceutical composition comprising an isolated Rspo1 protein and one or more pharmaceutically acceptable excipients in the manufacture of a medicament for treating diabetes in a subject in need thereof.

13. The use of claim 12, wherein the isolated Rspo1 protein is a human Rspo1.
14. The use of claim 12 or 13, wherein the isolated Rspo1 protein comprises an amino acid sequence having at least 90% identity with SEQ ID NO:3.
- 5 15. The use of claims 12 to 14, wherein the isolated Rspo1 protein is a fusion protein comprising an Fc region of an antibody.
- 10 16. The use of claim 15, wherein said Fc region of an antibody is fused to the human Rspo1 protein via a peptide linker.
17. The use of claims 12 to 16, wherein the diabetes is type 1 or diabetes type 2.