binding and activity in both ZNRF3 and LGR4 receptors are indicated and also reported in Figure 1.

There is still a need to further improve Rspo1-derived proteins for optimizing their developability properties and pharmacological properties for use as a biological medicament.

An object of the present disclosure is therefore to provide novel recombinant variants of R-spondin proteins for their use as a drug, e.g. in the treatment of diabetes.

SUMMARY

In one example, the present disclosure refers to a recombinant variant of R-spondin protein comprising the following FU1, FU2, TSP and BR domains, wherein

a. FU1 is a domain having at least 80% identity to any of the FU1 domains of human Rspo1, Rspo2, Rspo3 or Rspo4 as shown in SEQ ID NO: 5, 9, 13 and 17 respectively,

b. FU2 is a domain having at least 80% identity to any of the FU2 domains of human Rspo1, Rspo2, Rspo3 or Rspo4 as shown in SEQ ID NO: 6, 10, 14, and 18 respectively,

c. TSP is a domain having at least 80% identity to any of the TSP domains of human Rspo1, Rspo2, Rspo3 or Rspo4 as shown in SEQ ID NO: 7, 11, 15, and 19, respectively and,

d. BR is a domain having at least 80% identify to any of BR domains of human Rspo1, Rspo2, Rspo3, or Rspo4 as shown in SEQ ID NO: 8, 12, 16, and 20 respectively,

wherein said recombinant variant includes at least one amino acid substitution at position H108 or N109 of Rspo1 or corresponding residues in Rspo2, Rspo3 or Rspo4.

In another example, the present disclosure refers to an Fc fusion protein which includes a recombinant variant as disclosed herein, with an Fc fragment fused directly, or indirectly via a peptide linker, to said recombinant variant.

In yet another example, the present disclosure refers to use of the recombinant variant as disclosed herein or the fusion protein as disclosed herein, in the manufacture of a medicament for treating diabetes, more preferably diabetes type I or II.

In another example, the present disclosure refers to a nucleic acid encoding a recombinant variant as disclosed herein or a fusion protein as disclosed herein.

In yet another example, the present disclosure refers to a vector comprising a nucleic acid as disclosed herein.

In another example, the present disclosure refers to a host cell, comprising a nucleic acid as disclosed herein.

In yet another example, the present disclosure refers to a method for producing a recombinant variant as disclosed herein or a fusion protein as disclosed herein, comprising (i) culturing a host cell as disclosed herein under conditions for expression of said recombinant variant or fusion protein, (ii) recovering said recombinant variant or fusion protein, (iii) optionally purifying said recombinant variant or fusion protein.

In one aspect, the present disclosure refers to a Rspo1 recombinant variant comprising the following FU1, FU2, TSP and BR domains of Rspo1, wherein a. FU1 is a domain having at least 80% identity to the FU1 domain of human Rspo1, as shown in SEQ ID NO: 5, b. FU2 is a domain having at least 80% identity to the FU2 domain of human Rspo1, as shown in SEQ ID NO: 6, c. TSP is a domain having at least 80% identity to the TSP domain of human Rspo1, as shown in SEQ ID NO: 7, and d. BR is a domain having at least 80% identify to BR domain of human Rspo1, as shown in SEQ ID NO: 8, wherein said Rspo1 recombinant variant includes at least one amino acid substitution H108K or N109D of Rspo1 according to the numbering of amino acid residues in the sequence set forth in SEQ ID NO: 1, and wherein said Rspo1 recombinant variant induces the proliferation of beta-cells.

In another aspect, the present disclosure refers to an Fc fusion protein which includes a Rspo1 recombinant variant as disclosed herein, with an Fc fragment fused directly, or indirectly via a peptide linker, to said recombinant variant.

In another aspect, the present disclosure refers to use of the Rspo1 recombinant variant as disclosed herein or the Fc fusion protein as disclosed herein, in the manufacture of a medicament for treating diabetes, preferably diabetes type I or II.

In another aspect, the present disclosure refers to a nucleic acid encoding a Rspo1 recombinant variant as disclosed herein or a Fc fusion protein as disclosed herein.

In another aspect, the present disclosure refers to a vector comprising a nucleic acid as disclosed herein.

In another aspect, the present disclosure refers to a host cell, comprising a nucleic acid as disclosed herein or a vector as disclosed herein.

In another aspect, the present disclosure refers to a method for producing a Rspo1 recombinant variant as disclosed herein or a Fc fusion protein as disclosed herein, comprising (i) culturing a host cell as disclosed herein under conditions for expression of said recombinant variant or fusion protein, (ii) recovering said recombinant variant or fusion protein, (iii) optionally purifying said recombinant variant or fusion protein.

DETAILED DESCRIPTION

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 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 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

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.

Substitution refers to the replacement of a naturally occurring amino acid either with another naturally occurring amino acid or with an unnatural amino acid. For example, during chemical synthesis of a synthetic peptide, the native amino acid can be readily replaced by another naturally occurring amino acid or an unnatural amino acid.

CLAIMS

1. A Rspo1 recombinant variant comprising the following FU1, FU2, TSP and BR domains of Rspo1, wherein
    a. FU1 is a domain having at least 80% identity to the FU1 domain of human Rspo1, as shown in SEQ ID NO: 5,
    b. FU2 is a domain having at least 80% identity to the FU2 domain of human Rspo1, as shown in SEQ ID NO: 6,
    c. TSP is a domain having at least 80% identity to the TSP domain of human Rspo1, as shown in SEQ ID NO: 7, and
    d. BR is a domain having at least 80% identify to BR domain of human Rspo1, as shown in SEQ ID NO: 8,

    wherein said Rspo1 recombinant variant includes at least one amino acid substitution H108K or N109D of Rspo1 according to the numbering of amino acid residues in the sequence set forth in SEQ ID NO: 1, and wherein said Rspo1 recombinant variant induces the proliferation of beta-cells.

2. The Rspo1 recombinant variant according to claim 1, which has no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions in each of the FU1 or FU2 domain when aligned with corresponding human Rspo1 FU1 domain of SEQ ID NO:5 and Rspo1 FU2 domain of SEQ ID NO:6 respectively.

3. The Rspo1 recombinant variant according to claim 1 or 2, which comprises a deletion of residues 21-31 of Rspo1 according to the numbering of amino acid residues in the sequence set forth in SEQ ID NO: 1.

4. The Rspo1 recombinant variant according to any one of claims 1 to 3 which includes the following amino acid substitutions: H108K and N109D of Rspo1 according to the numbering of amino acid residues in the sequence set forth in SEQ ID NO: 1.

5. The Rspo1 recombinant variant according to any one of claims 1 to 4, comprising or essentially consisting of SEQ ID NO:66.

6. The Rspo1 recombinant variant according to any one of claims 1 to 4, which comprises at least an amino acid substitution of R66 in human Rspo1 FU1 domain of SEQ ID NO:5, said amino acid substitution decreasing or abolishing ZNRF3 binding, typically said variant comprises Rspo1 FU1 domain of SEQ ID NO:5 having a single amino acid substitution of residue R66 decreasing or abolishing ZNRF3 binding, typically R66A.

7. The Rspo1 recombinant variant according to any one of claims 1 to 4 or 6, wherein said variant does not comprise an N-glycosylation site between the FU2 and TSP domain, for example it includes a mutation in residue N137 of Rspo1, to suppress N-glycosylation at this position.

8. The Rspo1 recombinant variant according to any one of claims 1 to 7, which exhibits one or more of the following properties to a level at least similar to Rspo1 protein of SEQ ID NO: 41:
   (i) it binds to LGR4 receptor with at least the same affinity as reference human Rspo1 of SEQ ID NO:41, as measured in Rspo1/LGR4 binding affinity in vitro assay, for example as determined by SPR assay;
   (ii) it induces the proliferation of functional beta-cells to at least a similar level as reference human Rspo1 of SEQ ID NO:41, for example as determined in an in vitro Min6 beta-cell proliferation assay; and/or,
   (iii) it induces the proliferation of functional beta-cells to at least a similar level as reference human Rspo1 of SEQ ID NO:41, for example as determined in an in vivo beta-cell proliferation assay.

9. The Rspo1 recombinant variant according to claim 1, wherein

TSP is a domain having 100% identity to any of the TSP domains of human Rspo1, as shown in SEQ ID NO: 7;

BR is a domain having 100% identify to any of BR domains of human Rspo1, as shown in SEQ ID NO: 8;

FU1 is a domain having at least 80% identity to the FU1 domain of human Rspo1, as shown in SEQ ID NO: 5, and

FU2 is a domain having at least 80% identity to the FU2 domain of human Rspo1, as shown in SEQ ID NO: 6.

10. An Fc fusion protein which includes a Rspo1 recombinant variant according to any one of claims 1 to 9, with an Fc fragment fused directly, or indirectly via a peptide linker, to said recombinant variant.

11. The Fc fusion protein according to claim 10, wherein the Fc fragment comprises SEQ ID NO: 29.

12. The Fc fusion protein according to claim 10 or 11, wherein the Rspo1 recombinant variant comprises or essentially consists of SEQ ID NO: 66 and wherein the Fc fragment is fused directly, or indirectly via a peptide linker to the recombinant variant either at the C-terminal or N-terminal, preferably at the C-terminal.

13. Use of the Rspo1 recombinant variant according to any one of claims 1 to 9 or the Fc fusion protein according to any one of claims 10 to 12, in the manufacture of a medicament for treating diabetes, preferably diabetes type I or II.

14. A nucleic acid encoding a Rspo1 recombinant variant according to any one of claims 1 to 9 or a Fc fusion protein according to any one of claims 10 to 12.

15. A vector comprising a nucleic acid according to claim 14.

16. A host cell, comprising a nucleic acid according to claim 14 or a vector of claim 15.

17. A method for producing a Rspo1 recombinant variant according to any of claims 1 to 9 or a Fc fusion protein according to any one of claims 10 to 12, comprising (i) culturing a host cell of claim 16 under conditions for expression of said recombinant variant or fusion protein, (ii) recovering said recombinant variant or fusion protein, (iii) optionally purifying said recombinant variant or fusion protein.