Claims

1. A fusion protein comprising the polypeptide with structure A-B-C or C-B-A or B-A-C or B-C-A or A-C-B or C-A-B or A-B-C-B-C or A-C-B or A-B-C-B or A-C-B-C, wherein
A is a GLP-1R (glucagon-like peptide-1 receptor) agonist and
C is an FGF-21 (fibroblast growth factor 21) compound and
B is a linker comprising about 0 to 1000 amino acids.

2. The fusion protein according to claim 1, wherein the linker comprises a functional moiety conferring one or more additional functions beyond that of linking A and C.

3. The fusion protein according to claim 2, wherein the linker is a peptide linker.

4. The fusion protein according to claim 3, wherein the FGF-21 compound is selected from the group of native FGF-21, FGF-21 mimetic and SEQ ID NO: 3.

5. The fusion protein according to claim 4, wherein the FGF-21 mimetic is selected from a protein having at least about 80% amino acid sequence identity to the amino acid sequence shown in SEQ ID NO: 3 and having FGF-21 activity, a FGF-21 fusion protein and/or a FGF-21 conjugate

6. The fusion protein according to claim 4, wherein the FGF-21 mimetic is selected from a protein having at least about 90% amino acid sequence identity to the amino acid sequence shown in SEQ ID NO: 3 and having FGF-21 activity, a FGF-21 fusion protein and/or a FGF-21 conjugate

7. The fusion protein according to claim 4, wherein the FGF-21 mimetic is selected from a protein having at least about 96% amino acid sequence identity to the amino acid sequence shown in SEQ ID NO: 3 and having FGF-21 activity, a FGF-21 fusion protein and/or a FGF-21 conjugate.

8. The fusion protein according to claim 7, wherein the FGF-21 mimetic is selected from a FGF-21 mutein, a FGF-21-Fc fusion protein, a FGF-21-HSA fusion protein and/or a PEGylated FGF-21.

9. The fusion protein according to claim 8, wherein the GLP-1R agonist is selected from a bioactive GLP-1, a GLP-1 analogue or a GLP-1 substitute.

10. The fusion protein according to claim 9, wherein the GLP-1R agonist is selected from GLP-1(7-37), GLP-1(7-36)amide, exendin-4, liraglutide, CJC-1131, albugon, albiglutide, exenatide, exenatide-LAR, oxyntomodulin, lixisenatide, geniproside, or a short peptide with GLP-1R agonistic activity.

11. The fusion protein according to claims 10, wherein the linker comprises one or more of the following functional moieties a) to h):
a) a moiety conferring increased stability and/or half-life to the fusion such as an XTENylation or PASylation sequence or Elastin-like polypeptides (ELPs);
b) an entry site for covalent modification of the fusion protein such as a cysteine or lysine residue
c) a moiety with intra- or extracellular targeting function such as a protein-binding scaffold
d) a protease cleavage site such as a FactorXa cleavage site or a cleavage site for another extracellular protease;
e) a Fc portion of an immunoglobulin, e.g. the Fc portion of IgG4;
f) HSA;
g) an amino acid sequence comprising one or more histidine.
h) an albumin binding domain (ABD);

12. The fusion protein according to claim 11, wherein the linker consists of the one or more functional moieties.

13. The fusion protein according to claim 10, wherein the linker comprises additional amino acids in addition to the functional moiety.

14. The fusion protein according to claim 13, wherein the linker comprises one or more of the following protease cleavage sites:
a) a factor Xa cleavage site and preferably comprising or consisting of the sequence IEGR (SEQ ID NO:11)

b) a protease cleavage site and preferably comprising or consisting of at least one arginine and more preferably comprising or consisting of the sequence GGGRR (SEQ ID NO: 14).

15. The fusion protein according to claim 14, wherein the linker comprises or consists of an entry site for covalent modification and preferably comprising or consisting of the sequence according to SEQ ID NO:13.

16. The fusion protein according to claim 15, wherein the linker comprises or consists of a protein stabilisation sequence and preferably comprises a PASylation sequence selected from the group of: SEQ ID NO:12, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, and SEQ ID NO: 101. .

17. The fusion protein according to claim 16, wherein the linker comprises or consists of one or more entry sites for covalent modification of the fusion protein such as a cysteine or a lysine and preferably a cysteine.

18. The fusion protein according to claim 17, comprising one or more moieties D being covalently attached to the entry site(s) for covalent modification of the linker.

19. The fusion protein according to claim 18, wherein the covalently attached moiety or moieties D are selected from the list consisting of:
a) a targeting unit such as an antibody or protein-binding scaffold
b) a protein-stabilizing unit such as a hydroxyethyl starch derivative (HES) or a polyethylenglycol or derivative thereof (PEG or PEG derivative)
c) a fatty acid.

20. The fusion protein according to claim 19, comprising a tag for protein-purification such as a His-tag and wherein the tag is preferably N- or C-terminally attached to the fusion protein.

21. The fusion protein according to claim 20 comprising a protease cleavage site between the protein-purification tag and the remaining parts of the fusion protein, wherein the protease cleavage site is preferably a Sumo protease cleavage site.

22. The fusion protein according to claim 21, wherein A is an FGF-21 mutein and C is exenatide, exendin-4 or lixisenatide.

23. The fusion protein according to claim 22, wherein B has a sequence selected from the group of: SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13,SEQ ID NO:14, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, and SEQ ID NO: 101.

24. The fusion protein according to claim 23, wherein A is an FGF-21 mutein comprising or consisting of SEQ ID NO: 102.

25. The fusion protein according to claim 24, wherein C is exenatide.

26. The fusion protein according to claim 25 for use as a medicament.

27. A pharmaceutical composition comprising the fusion protein of claim 1 together with a pharmaceutically acceptable excipient.

28. A pharmaceutical composition comprising the fusion protein of claim 1 together with a pharmaceutically acceptable excipient for use as a medicament.

29. Article of manufacture comprising
a) a pharmaceutical composition according to claim 27 and
b) a container or packaging material.

30. A method of treating a disease or disorder of a patient, in which the increase of FGF-21 receptor autophosphorylation or in which the increase of FGF-21 efficacy is beneficial for the curing, prevention or amelioration of the disease or disorder, wherein the method comprises administration to the patient of a fusion protein of claim 1.

31. A method of treating a cardiovascular disease and/or diabetes mellitus and/or at least one metabolic syndrome which increases the risk of developing a cardiovascular

disease and/or diabetes mellitus, preferably Type 2-diabetes in a patient comprising the administration to the patient of a fusion protein of claim 1.

32. A method of lowering plasma glucose levels, of lowering the lipid content in the liver, of treating hyperlipidemia, of treating hyperglycemia, of increasing the glucose tolerance, of decreasing insulin tolerance, of increasing the body temperature, and/or of reducing weight of a patient comprising the administration to the patient of a fusion protein of claim.

33. A nucleic acid encoding the fusion protein according to claim 1, optionally comprising one of the following nucleic acid sequences:
a) a nucleic acid sequence according to one of the sequences with ID NOs: 27 to 38
b) a nucleic acid coding for a protein sequence according to SEQ ID NOs: 15 to 26 and 39 to 44
c) a nucleic acid hybridizing under stringent conditions with a nucleic acid according to a) or b).

34. A vector comprising the nucleic acid of claim 33 suitable for expression of the encoded protein in a eukaryotic or prokaryotic host.

35. A cell stably or transiently carrying the vector of claim 34 and capable of expressing the fusion protein according to claims 1 under appropriate culture conditions.

36. A method of preparing the fusion protein of claim 1 comprising
a) cultivating a culture of cells under appropriate culture conditions for the fusion protein to be expressed in the cell, or
b) harvesting or purifying the fusion protein from a culture comprising cells that have been cultivated under appropriate conditions for the fusion protein to be expressed, or
c) cultivating the cells according to step a) and purifying the fusion protein according to step b) and optionally
d) cleaving of a His-tag using a protease of fusion protein.

Claims

1. A fusion protein comprising the polypeptide with structure A-B-C or C-B-A or B-A-C or B-C-A or A-C-B or C-A-B or A-B-C-B-C or A-C-B or A-B-C-B or A-C-B-C, wherein
A is a GLP-1R (glucagon-like peptide-1 receptor) agonist and
C is an FGF-21 (fibroblast growth factor 21) compound and
B is a linker comprising about 0 to 1000 amino acids.

2. The fusion protein according to claim 1, wherein the linker comprises a functional moiety conferring one or more additional functions beyond that of linking A and C.

3. The fusion protein according to claim 2, wherein the linker is a peptide linker.

4. The fusion protein according to claim 3, wherein the FGF-21 compound is selected from the group of native FGF-21, FGF-21 mimetic and SEQ ID NO: 3.

5. The fusion protein according to claim 4, wherein the FGF-21 mimetic is selected from a protein having at least about 80% amino acid sequence identity to the amino acid sequence shown in SEQ ID NO: 3 and having FGF-21 activity, a FGF-21 fusion protein and/or a FGF-21 conjugate

6. The fusion protein according to claim 4, wherein the FGF-21 mimetic is selected from a protein having at least about 90% amino acid sequence identity to the amino acid sequence shown in SEQ ID NO: 3 and having FGF-21 activity, a FGF-21 fusion protein and/or a FGF-21 conjugate

7. The fusion protein according to claim 4, wherein the FGF-21 mimetic is selected from a protein having at least about 96% amino acid sequence identity to the amino acid sequence shown in SEQ ID NO: 3 and having FGF-21 activity, a FGF-21 fusion protein and/or a FGF-21 conjugate.

8. The fusion protein according to claim 7, wherein the FGF-21 mimetic is selected from a FGF-21 mutein, a FGF-21-Fc fusion protein, a FGF-21-HSA fusion protein and/or a PEGylated FGF-21.

9. The fusion protein according to claim 8, wherein the GLP-1R agonist is selected from a bioactive GLP-1, a GLP-1 analogue or a GLP-1 substitute.

10. The fusion protein according to claim 9, wherein the GLP-1R agonist is selected from GLP-1(7-37), GLP-1(7-36)amide, exendin-4, liraglutide, CJC-1131, albugon, albiglutide, exenatide, exenatide-LAR, oxyntomodulin, lixisenatide, geniproside, or a short peptide with GLP-1R agonistic activity.

11. The fusion protein according to claims 10, wherein the linker comprises one or more of the following functional moieties a) to h):
a) a moiety conferring increased stability and/or half-life to the fusion such as an XTENylation or PASylation sequence or Elastin-like polypeptides (ELPs);
b) an entry site for covalent modification of the fusion protein such as a cysteine or lysine residue
c) a moiety with intra- or extracellular targeting function such as a protein-binding scaffold
d) a protease cleavage site such as a FactorXa cleavage site or a cleavage site for another extracellular protease;
e) a Fc portion of an immunoglobulin, e.g. the Fc portion of IgG4;
f) HSA;
g) an amino acid sequence comprising one or more histidine.
h) an albumin binding domain (ABD);

12. The fusion protein according to claim 11, wherein the linker consists of the one or more functional moieties.

13. The fusion protein according to claim 10, wherein the linker comprises additional amino acids in addition to the functional moiety.

14. The fusion protein according to claim 13, wherein the linker comprises one or more of the following protease cleavage sites:
a) a factor Xa cleavage site and preferably comprising or consisting of the sequence IEGR (SEQ ID NO:11)

b) a protease cleavage site and preferably comprising or consisting of at least one arginine and more preferably comprising or consisting of the sequence GGGRR (SEQ ID NO: 14).

15. The fusion protein according to claim 14, wherein the linker comprises or consists of an entry site for covalent modification and preferably comprising or consisting of the sequence according to SEQ ID NO:13.

16. The fusion protein according to claim 15, wherein the linker comprises or consists of a protein stabilisation sequence and preferably comprises a PASylation sequence selected from the group of: SEQ ID NO:12, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, and SEQ ID NO: 101. .

17. The fusion protein according to claim 16, wherein the linker comprises or consists of one or more entry sites for covalent modification of the fusion protein such as a cysteine or a lysine and preferably a cysteine.

18. The fusion protein according to claim 17, comprising one or more moieties D being covalently attached to the entry site(s) for covalent modification of the linker.

19. The fusion protein according to claim 18, wherein the covalently attached moiety or moieties D are selected from the list consisting of:
a) a targeting unit such as an antibody or protein-binding scaffold
b) a protein-stabilizing unit such as a hydroxyethyl starch derivative (HES) or a polyethylenglycol or derivative thereof (PEG or PEG derivative)
c) a fatty acid.

20. The fusion protein according to claim 19, comprising a tag for protein-purification such as a His-tag and wherein the tag is preferably N- or C-terminally attached to the fusion protein.

21. The fusion protein according to claim 20 comprising a protease cleavage site between the protein-purification tag and the remaining parts of the fusion protein, wherein the protease cleavage site is preferably a Sumo protease cleavage site.

22. The fusion protein according to claim 21, wherein A is an FGF-21 mutein and C is exenatide, exendin-4 or lixisenatide.

23. The fusion protein according to claim 22, wherein B has a sequence selected from the group of: SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13,SEQ ID NO:14, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, and SEQ ID NO: 101.

24. The fusion protein according to claim 23, wherein A is an FGF-21 mutein comprising or consisting of SEQ ID NO: 102.

25. The fusion protein according to claim 24, wherein C is exenatide.

26. The fusion protein according to claim 25 for use as a medicament.

27. A pharmaceutical composition comprising the fusion protein of claim 1 together with a pharmaceutically acceptable excipient.

28. A pharmaceutical composition comprising the fusion protein of claim 1 together with a pharmaceutically acceptable excipient for use as a medicament.

29. Article of manufacture comprising
a) a pharmaceutical composition according to claim 27 and
b) a container or packaging material.

30. Use of a fusion protein of claim 1 for the manufacture of a medicament for treating a disease or disorder of a patient, in which the increase of FGF-21 receptor autophosphorylation or in which the increase of FGF-21 efficacy is beneficial for the curing, prevention or amelioration of the disease or disorder.

31. Use of a fusion protein of claim 1 for the manufacture of a medicament for treating a cardiovascular disease and/or diabetes mellitus and/or at least one metabolic syndrome

which increases the risk of developing a cardiovascular disease and/or diabetes mellitus, preferably Type 2-diabetes.

32. Use of a fusion protein of claim 1for the manufacture of a medicament for lowering plasma glucose levels, for lowering the lipid content in the liver, for treating hyperlipidemia, for treating hyperglycemia, for increasing the glucose tolerance, for decreasing insulin tolerance, for increasing the body temperature, and/or for reducing weight of a patient .

33. A nucleic acid encoding the fusion protein according to claim 1, optionally comprising one of the following nucleic acid sequences:
a) a nucleic acid sequence according to one of the sequences with ID NOs: 27 to 38
b) a nucleic acid coding for a protein sequence according to SEQ ID NOs: 15 to 26 and 39 to 44
c) a nucleic acid hybridizing under stringent conditions with a nucleic acid according to a) or b).

34. A vector comprising the nucleic acid of claim 33 suitable for expression of the encoded protein in a eukaryotic or prokaryotic host.

35. A cell stably or transiently carrying the vector of claim 34 and capable of expressing the fusion protein according to claims 1 under appropriate culture conditions.

36. A method of preparing the fusion protein of claim 1 comprising
a) cultivating a culture of cells under appropriate culture conditions for the fusion protein to be expressed in the cell, or
b) harvesting or purifying the fusion protein from a culture comprising cells that have been cultivated under appropriate conditions for the fusion protein to be expressed, or
c) cultivating the cells according to step a) and purifying the fusion protein according to step b) and optionally
d) cleaving of a His-tag using a protease of fusion protein.