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[Summary]
[Technical Problem to be solved]
[0012]

The present inventors have thought that the techniques of dissociating, by protease cleavage, a masking peptide inhibiting the antigen binding activity of an antibody so that the antibody restores its antigen binding activity, as described above might cause adverse reactions, because the antibody cleaved at a lesion site may distribute to normal tissues through blood flow, as the cleavage by protease is irreversible.

The present invention has been made on the basis of such an idea. An object of the present invention is to provide a pharmaceutical composition useful in disease treatment with a reduced adverse reaction, and an active ingredient thereof. Another object of the present invention is to provide methods for screening for and producing the pharmaceutical composition and the active ingredient.

[Solution to Problem]
[0013]

The present inventors have conducted diligent studies and consequently developed a polypeptide comprising an antigen binding domain and a carrying moiety having an inhibiting domain that inhibits the binding activity of the antigen binding domain, and having a longer half-life than the half-life of the antigen binding domain which exists alone. It is considered that use of the polypeptide can allow the antigen binding domain to restore its antigen binding activity in a disease tissue and exert the antigen binding activity in the disease tissue. Furthermore, the systemic distribution of an activated form of the antigen binding domain can be suppressed owing to the difference in half-life between the polypeptide comprising the antigen binding domain whose antigen binding activity is inhibited and a polypeptide comprising the antigen binding domain whose antigen binding activity is restored. Moreover, the present inventors have found that the polypeptide or a pharmaceutical composition comprising the polypeptide is useful in disease treatment and also found that: the polypeptide or the pharmaceutical composition is useful in disease treatment which involves administering the polypeptide; and the polypeptide is useful in the production of a drug for disease treatment. The present inventors have further developed methods for screening for and producing the polypeptide, methods for producing and screening for a single-domain antibody whose antigen binding activity can be inhibited by associating with particular VL, VH or VHH, and a library including a single-domain antibody whose antigen binding activity can be inhibited by associating with particular VL, VH or VHH, completing the present disclosure.

[0013a] In one aspect, the invention refers to a polypeptide comprising an antigen binding domain and a carrying moiety, the carrying moiety having an inhibiting domain that inhibits the antigen binding activity of the antigen binding domain, and the antigen binding domain having a shorter half-life in blood than that of the carrying moiety.

[0013b] In another aspect, the invention refers to a pharmaceutical composition comprising the polypeptide as described herein.

[0013c] In another aspect, the invention refers to a host cell comprising a polynucleotide encoding the polypeptide as described herein, or a vector comprising the polynucleotide as described herein.

[0013d] In another aspect, the invention refers to a method for producing the polypeptide as described herein.

[0013e] In another aspect, the invention refers to a method for screening for a single-domain antibody whose antigen binding activity can be inhibited by associating with particular VL, associating with particular VH, or associating with particular VHH.

[0013f] In another aspect, the invention refers to a method for producing a single-domain antibody whose antigen binding activity is inhibited by associating with particular VL, associating with particular VH, or associating with particular VHH.

[0013g] In another aspect, the invention refers to a library comprising a plurality of fusion polypeptides of single-domain antibodies each linked to a first association sustaining domain, wherein the single-domain antibodies include a single-domain antibody whose antigen binding activity can be inhibited or lost by associating with particular VL, a single-domain antibody whose antigen binding activity can be inhibited or lost by associating with particular VH, or a single-domain antibody whose antigen binding activity can be inhibited or lost by associating with particular VHH.

[0013h] In another aspect, the invention refers to a method for screening a library as described herein for a fusion polypeptide comprising a single-domain antibody whose antigen binding activity can be inhibited or could lost by associating with particular VL, a single-domain antibody whose antigen binding activity can be inhibited or lost by associating with particular VH, or a single-domain antibody whose antigen binding activity can be inhibited or lost by associating with particular VHH.

[0014]

The present disclosure is based on these findings and specifically encompasses exemplary examples described below.

(1) A polypeptide comprising an antigen binding domain and a carrying moiety, the carrying moiety having an inhibiting domain that inhibits the antigen binding activity of the antigen binding domain, and the antigen binding domain having a shorter half-life in blood than that of the carrying moiety.

(2) The polypeptide according to (1), wherein the molecular weight of the antigen binding domain is smaller than that of the carrying moiety.

(3) The polypeptide according to (1) or (2), wherein the molecular weight of the antigen binding domain is 60 kDa or smaller.

(4) The polypeptide according to any of (1) to (3), wherein the carrying moiety has FcRn binding activity, and the antigen binding domain has no FcRn binding activity or has weaker FcRn binding activity than that of the carrying moiety.

(5) The polypeptide according to any of (1) to (4), wherein the antigen binding domain is capable of being released from the polypeptide, and the antigen binding domain released from the polypeptide has higher antigen binding activity than that before the release

CLAIMS

1. A polypeptide comprising an antigen binding domain and a carrying moiety, the carrying moiety having an inhibiting domain that inhibits the antigen binding activity of the antigen binding domain, and the antigen binding domain having a shorter half-life in blood than that of the carrying moiety.

2. The polypeptide according to claim 1, wherein the molecular weight of the antigen binding domain is smaller than that of the carrying moiety.

3. The polypeptide according to claim 1 or 2, wherein the molecular weight of the antigen binding domain is 60 kDa or smaller.

4. The polypeptide according to any one of claims 1 to 3, wherein the carrying moiety has FcRn binding activity, and the antigen binding domain has no FcRn binding activity or has weaker FcRn binding activity than that of the carrying moiety.

5. The polypeptide according to any one of claims 1 to 4, wherein the antigen binding domain is capable of being released from the polypeptide, and the antigen binding domain released from the polypeptide has higher antigen binding activity than that before the release.

6. The polypeptide according to any one of claims 1 to 5, wherein the inhibiting domain of the carrying moiety associates with the antigen binding domain and thereby inhibits the antigen binding activity of the antigen binding domain.

7. The polypeptide according to claim 5 or 6, wherein the polypeptide comprises a cleavage site, wherein the cleavage site is cleaved so that the antigen binding domain becomes capable of being released from the polypeptide, or/and the association of the inhibiting domain of the carrying moiety with the antigen binding domain is canceled.

8. The polypeptide according to claim 7, wherein the cleavage site comprises a protease cleavage sequence.

9. The polypeptide according to claim 8, wherein the protease is a target tissue specific protease.

10. The polypeptide according to any of claims 1 to 9, wherein the antigen binding domain comprises a single-domain antibody or is a single-domain antibody, wherein the inhibiting domain of the carrying moiety inhibits the antigen binding activity of the single-domain antibody.

11. The polypeptide according to any of claims 1 to 10, wherein the antigen binding domain comprises a single-domain antibody, and the inhibiting domain of the carrying moiety is VHH, antibody VH, or antibody VL, wherein the antigen binding activity of the single-domain antibody is inhibited by the VHH, or the antibody VH, or the antibody VL.

12. The polypeptide according to any of claims 1 to 11, wherein the carrying moiety comprises an antibody constant region.

13. The polypeptide according to claim 12, wherein the polypeptide has a protease cleavage sequence, wherein the protease cleavage sequence is located near the boundary between the antigen binding domain and the antibody constant region.

14. A pharmaceutical composition comprising the polypeptide of any of claims 1 to 13.

15. A host cell comprising a polynucleotide encoding the polypeptide according to any one of claims 1 to 13, or a vector comprising the polynucleotide aforementioned.

16. A method for producing the polypeptide of any one of claims 1 to 13.

17. A method for screening for a single-domain antibody whose antigen binding activity can be inhibited by associating with particular VL, associating with particular VH, or associating with particular VHH.

18. A method for producing a single-domain antibody whose antigen binding activity is inhibited by associating with particular VL, associating with particular VH, or associating with particular VHH.

19. A library comprising a plurality of fusion polypeptides of single-domain

antibodies each linked to a first association sustaining domain, wherein the single-domain antibodies include a single-domain antibody whose antigen binding activity can be inhibited or lost by associating with particular VL, a single-domain antibody whose antigen binding activity can be inhibited or lost by associating with particular VH, or a single-domain antibody whose antigen binding activity can be inhibited or lost by associating with particular VHH.

20. A method for screening a library according to claim 19 for a fusion polypeptide comprising a single-domain antibody whose antigen binding activity can be inhibited or could lost by associating with particular VL, a single-domain antibody whose antigen binding activity can be inhibited or lost by associating with particular VH, or a single-domain antibody whose antigen binding activity can be inhibited or lost by associating with particular VHH.