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[Summary]

[0011a]

In one aspect, the present disclosure refers to a polypeptide comprising an antigen-binding domain and a carrying moiety, wherein the carrying moiety has an inhibiting domain that inhibits the antigen-binding activity of the antigen-binding domain, and wherein the polypeptide has a protease cleavage sequence comprising one or a plurality of sequences selected from the sequences of SEQ ID NOs: 678, 171, 801, 492, 802, 493,168-170, 172-177, 488-491, 494, 500-506, 854-899, 901-913, 916-919, 921-951, wherein the antigen-binding domain comprises a single-domain antibody or is a single-domain antibody, and the inhibiting domain of the carrying moiety is a VHH, an antibody VH, or an antibody VL,
wherein the antigen-binding activity of the antigen-binding domain is inhibited by the association of the inhibiting domain of the carrying moiety with the antigen-binding domain, wherein inhibition of antigen-binding activity of the antigen-binding domain by the inhibiting domain in a state where the protease cleavage sequence has been cleaved by a protease is weaker than the inhibition of antigen-binding activity of the antigen-binding domain by the inhibiting domain in a state where the protease cleavage sequence is uncleaved.

[0011b]

In another aspect, the present disclosure refers to a pharmaceutical composition comprising the polypeptide as disclosed herein.

[0011c]

In yet another aspect, the present disclosure refers to a method for producing the polypeptide as disclosed herein.

[Technical Problem]

[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 side effects, 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 disclosure has been made on the basis of such an idea. An object of the present disclosure is to provide a pharmaceutical composition useful in disease treatment with less side effects, and an active ingredient thereof. Another object of the disclosure 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 polypeptides 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(s) and exert the antigen-binding activity in the disease tissue(s). Furthermore, the systemic distribution of an activated form of the antigen-binding domain can be suppressed

[CLAIMS]

1. A polypeptide comprising an antigen-binding domain and a carrying moiety, wherein the carrying moiety has an inhibiting domain that inhibits the antigen-binding activity of the antigen-binding domain, and wherein the polypeptide has a protease cleavage sequence comprising one or a plurality of sequences selected from the sequences of SEQ ID NOs: 678, 171, 801, 492, 802, 493, 168-170, 172-177, 488-491, 494, 500-506, 854-899, 901-913, 916-919, 921-951, wherein the antigen-binding domain comprises a single-domain antibody or is a single-domain antibody, and the inhibiting domain of the carrying moiety is a VHH, an antibody VH, or an antibody VL,
wherein the antigen-binding activity of the antigen-binding domain is inhibited by the association of the inhibiting domain of the carrying moiety with the antigen-binding domain, wherein inhibition of antigen-binding activity of the antigen-binding domain by the inhibiting domain in a state where the protease cleavage sequence has been cleaved by a protease is weaker than the inhibition of antigen-binding activity of the antigen-binding domain by the inhibiting domain in a state where the protease cleavage sequence is uncleaved.

2. The polypeptide of claim 1, wherein the protease cleavage sequence is located between the carrying moiety and the antigen binding domain, and wherein the protease cleavage sequence is cleaved so that the association of the inhibiting domain of the carrying moiety with the antigen binding domain is canceled.

3. The polypeptide of claim 1 or 2, wherein the antigen-binding domain has a shorter half-life in blood than the carrying moiety.

4. The polypeptide of any one of claims 1 to 3, wherein the molecular weight of the antigen binding domain is 60kDa or smaller.

5. The polypeptide of any one of claims 1 to 4, 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.

6. The polypeptide of any one of claims 1 to 5, wherein the antigen-binding domain is capable of being released from the polypeptide, and wherein the antigen-binding domain has higher antigen-binding activity in a state where it is released from the polypeptide than antigen-binding activity in a state where it is not released from the polypeptide.

7. The polypeptide of claim 6 , wherein the protease cleavage sequence is cleaved by a protease, so that the antigen-binding domain becomes capable of being released from the polypeptide.

8. The polypeptide of any one of claims 1 to 7, wherein the protease cleavage sequence is cleaved by a cancer tissue specific protease or an inflammatory tissue specific protease.

9. The polypeptide of any one of claims 1 to 8, wherein the antigen-binding activity of the single-domain antibody is inhibited by the VHH, the antibody VH, or the antibody VL.

10. The polypeptide of any one of claims 1 to 9, wherein the carrying moiety comprises an antibody constant region.

11. The polypeptide of claim 10, wherein the N terminus of the antibody constant region of the carrying moiety and the C terminus of the antigen-binding domain are fused *via* a linker or without a linker.

12. The polypeptide of claim 10 or 11, wherein the antibody binding domain is a single-domain antibody, wherein the protease cleavage sequence is located between the amino acid of position 101 (Kabat numbering) of the single-domain antibody and the amino acid of position 140 (EU numbering) of the antibody heavy chain constant region or the amino acid of position 101 (Kabat numbering) of the single-domain antibody and the amino acid of position 130 (EU numbering) of the antibody light chain constant region.

13. The polypeptide of claim 10 to 12, wherein the antibody constant region of the polypeptide is an IgG antibody constant region.

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

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