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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: 673, 371, 678, 171, 693, 389, 801, 813, 492, 1054, 802, 814, 493, and 833 to 852 and SEQ ID NOs: 1062 to 1081 and the sequences described in Table 1.

[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.

5 [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-
10 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

owing to the difference in half-lives 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 polypeptides or pharmaceutical compositions comprising the polypeptide are useful in disease treatment and also found that the polypeptides or the pharmaceutical compositions are useful in disease treatment which involves administering the polypeptide; and that the polypeptides are 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 its association with particular VL, VH or VHH, and libraries including a single-domain antibody whose antigen-binding activity can be inhibited by its association with particular VL, VH or VHH, and completed the present disclosure.

[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.

(6) The polypeptide according to any of (1) to (5), wherein the inhibiting domain of the carrying moiety is associated with the antigen-binding domain and thereby inhibits the antigen-binding activity of the antigen-binding domain.

(7) The polypeptide according to (5), 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.

(a) allowing the fusion polypeptides of the library to be displayed *in vitro*;

(b) providing an association partner of a second association sustaining domain fused with a particular VHH;

5 (c) selecting a fusion polypeptide(s) comprising a single-domain antibody that binds to the antigen or has antigen-binding activity of a predetermined value or higher; and

(d) allowing the fusion polypeptide(s) thus selected in the step (c) to be associated with the association partner provided in the step (b) and selecting a fusion polypeptide that does not bind to the antigen or has antigen-binding activity of a predetermined value or lower in a state where the single-domain antibody is associated with the VHH.

10 (140) The screening method according to (139), wherein the step (d) comprises allowing the fusion polypeptides selected in the step (c) to be displayed again *in vitro*.

(141) The screening method according to (139), wherein the step (c) comprises allowing the fusion polypeptide(s) to be associated only with the second association sustaining domain or confirming the antigen binding of the single-domain antibody contained in the fusion
15 polypeptide associated only with the second association sustaining domain.

(142) The screening method according to any of (133) to (141), wherein the step of allowing the fusion polypeptide(s) to be associated with the association partner in the step (d) is the step of allowing the association partner and the fusion polypeptides to be displayed together.

20 (143) The screening method according to any of (133) to (142), wherein the first association sustaining domain comprises an IgG antibody CH1 domain, and the second association sustaining domain comprises an antibody light chain constant region.

(144) The screening method according to any of (133) to (142), wherein the first association sustaining domain comprises an antibody light chain constant region, and the second association sustaining domain comprises an IgG antibody CH1 domain.

25 [0015]

Specifically, the present disclosure may also encompass the following exemplary examples.

30 (B1) 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: 833 to 852 and SEQ ID NOs: 1062 to 1081 and the sequences described in Table 1.

35 (B2) The polypeptide according to (B1), 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

[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: 673, 371, 678, 171, 693, 389, 801, 813, 492, 1054, 802, 814, 493, and 833 to 852 and SEQ ID NOs: 1062 to 1081 and the sequences described in Table 1.
2. The polypeptide of claim 1, 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.
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 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.
5. The polypeptide of any one of claims 1 to 4, 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.
6. The polypeptide of claim 4 or 5, wherein the protease cleavage sequence is cleaved by a protease, so that the antigen-binding domain becomes capable of being released from the polypeptide or/and so that the association of the inhibiting domain of the carrying moiety with the antigen-binding domain is canceled.
7. The polypeptide of any one of claims 1 to 6, wherein the protease is a cancer tissue specific protease or an inflammatory tissue specific protease.

8. The polypeptide of any one of claims 1 to 7, wherein the antigen-binding domain comprises a single-domain antibody or is a single-domain antibody, and wherein the inhibiting domain of the carrying moiety inhibits the antigen-binding activity of the single-domain antibody.
- 5 9. The polypeptide of any one of claims 1 to 8, wherein the antigen-binding domain comprises a single-domain antibody, wherein the inhibiting domain of the carrying moiety is a VHH, an antibody VH, or an antibody VL, and wherein the antigen-binding activity of the single-domain antibody is inhibited by the VHH, the antibody VH, or the antibody VL.
- 10 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
15 without a linker, and wherein the protease cleavage sequence is located near the boundary between the antigen-binding domain and the antibody constant region.
12. The polypeptide of claim 10 or 11, wherein the antibody constant region of the polypeptide is an IgG antibody constant region.
- 20 13. The polypeptide of any one of claims 1 to 12, wherein the polypeptide is an IgG antibody-like molecule.
14. A pharmaceutical composition comprising the polypeptide of any one of claims 1 to 13.
- 25 15. A method for producing the polypeptide of any one of claims 1 to 13.