

pharmaceutical composition is useful in the treatment of a disease which involves administering the ligand binding molecule; and the ligand binding molecule is useful in the production of a drug for the treatment of a disease. The present inventors have also developed a method for producing the ligand binding molecule, completing the present disclosure.

[0009a]

In one aspect, the present disclosure refers to a ligand binding molecule which is a molecule capable of binding to a ligand, wherein the molecule is a polypeptide having at least one protease cleavage sequence, and the ligand binding of the molecule cleaved at the at least one protease cleavage sequence is attenuated, wherein the antibody VL and the antibody VH in the ligand binding molecule are associated with each other, wherein the association is canceled by the cleavage of the protease cleavage sequence, wherein the protease cleavage sequence is: (1) located near the boundary between the antibody constant region and the antibody VH or/and near the boundary between the antibody constant region and the antibody VL; or (2) located on the variable region side with respect to amino acid position 140 (EU numbering) in an antibody heavy chain constant region; or (3) located on the variable region side with respect to amino acid position 130 (Kabat numbering position 130) in an antibody light chain constant region; or (4) inserted at a position of residues constituting a loop structure in the antibody VH or the antibody VL, and residues close to the loop structure. In another aspect, the present disclosure refers to a fusion protein of a ligand binding molecule as described herein fused with a ligand. In another aspect, the present disclosure refers to a polynucleotide encoding a ligand binding molecule as described herein or a fusion protein as described herein. In yet another aspect, the present disclosure refers to a host cell comprising a polynucleotide of the present disclosure, or a vector comprising the polynucleotide of the present disclosure. In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising a ligand binding molecule of the present disclosure. In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising a ligand binding molecule of the present disclosure and a ligand. In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising a fusion protein of the present disclosure. In yet another aspect, the present disclosure refers to a method for producing a ligand binding molecule of the present disclosure, or a fusion protein of the present disclosure.

[0010]

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

- (1) A ligand binding molecule which is a molecule capable of binding to a ligand, wherein the molecule is a polypeptide having at least one cleavage site, and the ligand binding of the molecule cleaved at the at least one cleavage site is attenuated.
- (2) The ligand binding molecule according to (1), wherein the ligand is released from the ligand binding molecule cleaved at the cleavage site.
- (3) The ligand binding molecule according to (1) or (2), wherein the cleavage site comprises a protease cleavage sequence.
- (4) The ligand binding molecule according to (3), wherein the protease is a target tissue specific protease.
- (5) The ligand binding molecule according to (4), wherein the target tissue is a cancer tissue, and the target tissue specific protease is a cancer tissue specific protease.
- (6) The ligand binding molecule according to (4), wherein the target tissue is an inflammatory tissue, and the target tissue specific protease is an inflammatory tissue specific protease.
- (7) The ligand binding molecule according to any of (3) to (6), wherein the protease is at least one protease selected from matriptase, urokinase (uPA), and metalloproteinase.
- (8) The ligand binding molecule according to (3), wherein the protease cleavage sequence is a sequence comprising a sequence selected from the sequences represented by SEQ ID NOs: 3, 34, 66, 70, 71, 72, 73, 35, 75, 76, and 345.
- (9) The ligand binding molecule according to any of (3) to (8), wherein a first flexible linker is further attached to one end of the protease cleavage sequence.
- (10) The ligand binding molecule according to (9), wherein a second flexible linker is further attached to the other end of the protease cleavage sequence.
- (11) The ligand binding molecule according to (9), wherein the first flexible linker is a flexible linker consisting of a glycine-serine polymer.
- (12) The ligand binding molecule according to (10), wherein the second flexible linker is a flexible linker consisting of a glycine-serine polymer.
- (13) The ligand binding molecule according to any of (1) to (12), wherein the ligand binding molecule comprises antibody VH, antibody VL, and an antibody constant region.

CLAIMS

1. A ligand binding molecule which is a molecule capable of binding to a ligand, wherein the molecule is a polypeptide having at least one protease cleavage sequence, and the ligand binding of the molecule cleaved at the at least one protease cleavage sequence is attenuated, wherein the ligand binding molecule comprises antibody VH, antibody VL, and an antibody constant region, wherein the antibody VL and the antibody VH in the ligand binding molecule are associated with each other, wherein the association is canceled by the cleavage of the protease cleavage sequence, wherein the protease cleavage sequence is:
 - (1) located near the boundary between the antibody constant region and the antibody VH or/and near the boundary between the antibody constant region and the antibody VL; or
 - (2) located on the variable region side with respect to amino acid position 140 (EU numbering) in an antibody heavy chain constant region; or
 - (3) located on the variable region side with respect to amino acid position 130 (Kabat numbering position 130) in an antibody light chain constant region; or
 - (4) inserted at a position of residues constituting a loop structure in the antibody VH or the antibody VL, and residues close to the loop structure.
2. The ligand binding molecule according to claim 1, wherein the protease is target tissue specific protease.
3. The ligand binding molecule according to claim 1 or 2, wherein the ligand is a molecule having biological activity, and the ligand binding molecule inhibits the biological activity of the ligand by binding to the ligand.
4. The ligand binding molecule according to any of claims 1 to 3, wherein the ligand is a cytokine or a chemokine.

5. The ligand binding molecule according to any of claims 1 to 4, wherein the ligand binding molecule is an IgG antibody.
6. The ligand binding molecule according to any of claims 1 to 5, wherein the ligand binding molecule is bound with the ligand.
7. The ligand binding molecule according to any of claims 1 to 5, wherein the ligand binding molecule is fused with the ligand.
8. A fusion protein of a ligand binding molecule according to any of claims 1 to 5 fused with a ligand.
9. The fusion protein according to claim 8, wherein the ligand binding molecule fused with the ligand does not further bind to another ligand.
10. The fusion protein according to claim 8 or 9, wherein the ligand binding molecule is fused with the ligand via a linker.
11. A polynucleotide encoding a ligand binding molecule according to any of claims 1 to 7 or a fusion protein according to any of claims 8 to 10.
12. A host cell comprising a polynucleotide according to claim 11, or a vector comprising the polynucleotide aforementioned.
13. A pharmaceutical composition comprising a ligand binding molecule according to any of claims 1 to 7.
14. A pharmaceutical composition comprising a ligand binding molecule according to any of claims 1 to 6 and a ligand.

15. A pharmaceutical composition comprising a fusion protein according to any of claims 8 to 10.
16. A method for producing a ligand binding molecule according to any of claims 1 to 7, or a fusion protein according to any of claims 8 to 10.