

However, there is a problem that upon co-expression during a process of producing a bispecific antibody, mispairing of antibody heavy and light chains of different specificities results in a number of non-functional byproducts. Therefore, to
5 solve this problem, there is a need for development of a technique for producing a desired multi-specific antibody construct in clinically sufficient amount and purity.

Disclosure of Invention

As a result of conducting intensive studies to solve the problems of existing
10 bispecific antibodies, the present inventors have developed a fusion protein, in which heavy and light chains of antibodies are linked through linkers, and thus are included as a single chain, and a bispecific or trispecific antibody comprising the same; and have found that these fusion proteins and antibodies solve the problem of mispairing and have a high killing effect on tumor cells.

15 Aspects of the invention are defined in the independent claims. Some optional features of the invention are defined in the dependent claims.

Accordingly, it may be possible to provide a fusion protein including an anti-mesothelin antibody, an anti-CD3 antibody, or an anti-EGFR antibody, and a bispecific or trispecific antibody comprising the same, and pharmaceutical compositions and
20 medicaments for treating cancer .

In one exemplary technique, there is provided a first fusion protein in the form of a single chain, including an antibody that specifically binds to mesothelin and an antibody that specifically binds to CD3.

25 In another exemplary technique, there is provided a second fusion protein in the form of a single chain, including an antibody that specifically binds to mesothelin.

In yet another exemplary technique, there is provided a third fusion protein in

the form of a single chain, including an antibody that specifically binds to EGFR.

In still yet another exemplary technique, there is provided a bispecific antibody, comprising the first fusion protein and the second fusion protein.

In still yet another exemplary technique, there is provided a trispecific antibody,
5 comprising the first fusion protein and the third fusion protein.

In still yet another exemplary technique, there is provided a pharmaceutical composition for treating cancer, comprising, as an active ingredient, the fusion protein, the bispecific antibody, or the trispecific antibody.

In still yet another exemplary technique, there is provided a use of the fusion
10 protein, the bispecific antibody, or the trispecific antibody.

In still yet another exemplary technique, there is provided a method for treating cancer, comprising a step of administering, to an individual, a therapeutically effective amount of the pharmaceutical composition for treating cancer.

15 **Advantageous Effects**

The fusion protein, and the bispecific antibody or the trispecific antibody comprising the same, as disclosed herein, can be produced in high yield and purity, and can be effectively used for cancer treatment due to their excellent tumor killing and growth inhibitory effects.

20

Brief Description of Drawings

Aspects of the invention will now be described, by way of example only, and with reference to the accompanying drawings in which:

FIG. 1 illustrates an exemplary schematic diagram of an anti-MSLN/anti-CD3
25 bispecific antibody (hereinafter referred to as HMI323/HMI323-A15).

FIG. 2 illustrates an exemplary schematic diagram of an anti-EGFR/anti-

MSLN/anti-CD3 trispecific antibody (hereinafter referred to as Cetuximab/HMI323-A15).

5

10

15

20

25

Claims

[Claim 1]

A first fusion protein having Structural Formula 1:

[Structural Formula 1]

N'-A-B-L1-C-D-L2-E-F-L3-G-H-L4-I-C'

wherein N' is the N-terminus of the fusion protein,

C' is the C-terminus of the fusion protein,

A is a light chain variable domain of an antibody that specifically binds to mesothelin, the domain including light chain CDR1 of SEQ ID NO: 25, light chain CDR2 of SEQ ID NO: 26, and light chain CDR3 of SEQ ID NO: 27,

B is a light chain constant domain of the antibody,

C is a heavy chain variable domain of the antibody that specifically binds to mesothelin, the domain including heavy chain CDR1 of SEQ ID NO: 28, heavy chain CDR2 of SEQ ID NO: 29, and heavy chain CDR3 of SEQ ID NO: 30,

D is a CH1 domain in heavy chain constant domains of the antibody,

E is a light chain variable domain of an antibody that specifically binds to CD3, the domain including light chain CDR1 of SEQ ID NO: 31, light chain CDR2 of SEQ ID NO: 32, and light chain CDR3 of SEQ ID NO: 33,

F is a light chain constant domain of the antibody,

G is a heavy chain variable domain of the antibody that specifically binds to CD3, the domain including heavy chain CDR1 of SEQ ID NO: 34, heavy chain CDR2 of SEQ ID NO: 35, and heavy chain CDR3 of SEQ ID NO: 36,

H is a CH1 domain in heavy chain constant domains of the antibody,

I is an Fc region or a variant thereof, and

each of L1, L2, L3, and L4 is a peptide linker.

[Claim 2]

The first fusion protein of claim 1, wherein each of L1, L2, L3, and L4 is a peptide linker consisting of 1 to 50 amino acids.

[Claim 3]

The first fusion protein of claim 1, wherein each of L1 and L3 is a peptide linker consisting of the amino acid sequence of SEQ ID NO: 43.

[Claim 4]

The first fusion protein of claim 1, wherein L2 is a peptide linker consisting of the amino acid sequence of SEQ ID NO: 44.

[Claim 5]

The first fusion protein of claim 1, wherein L4 is a peptide linker consisting of the amino acid sequence of SEQ ID NO: 45.

[Claim 6]

The first fusion protein of claim 1, wherein the first fusion protein consists of the amino acid sequence of SEQ ID NO: 1.

[Claim 7]

A second fusion protein having Structural Formula 2:

[Structural Formula 2]

N'-A-B-L1-C-D-L5-J-C'

wherein N' is the N-terminus of the fusion protein,

C' is the C-terminus of the fusion protein,

A is a light chain variable domain of an antibody that specifically binds to mesothelin, the domain including light chain CDR1 of SEQ ID NO: 25, light chain

CDR2 of SEQ ID NO: 26, and light chain CDR3 of SEQ ID NO: 27,

B is a light chain constant domain of the antibody,

C is a heavy chain variable domain of the antibody that specifically binds to mesothelin, the domain including heavy chain CDR1 of SEQ ID NO: 28, heavy chain CDR2 of SEQ ID NO: 29, and heavy chain CDR3 of SEQ ID NO: 30,

D is a CH1 domain in heavy chain constant domains of the antibody,

J is an Fc region or a fragment thereof, and

each of L1 and L5 is a peptide linker.

[Claim 8]

The second fusion protein of claim 7, wherein each of L1 and L5 is a peptide linker consisting of 1 to 50 amino acids.

[Claim 9]

The second fusion protein of claim 7, wherein L1 is a peptide linker consisting of the amino acid sequence of SEQ ID NO: 43.

[Claim 10]

The second fusion protein of claim 7, wherein L5 is a peptide linker consisting of the amino acid sequence of SEQ ID NO: 45.

[Claim 11]

The second fusion protein of claim 7, wherein the second fusion protein consists of the amino acid sequence of SEQ ID NO: 3.

[Claim 12]

A third fusion protein having Structural Formula 3:

[Structural Formula 3]

N'-K-M-L6-N-O-L7-P-C'

wherein N' is the N-terminus of the fusion protein,

C' is the C-terminus of the fusion protein,

K is a light chain variable domain of an antibody that specifically binds to EGFR, the domain including light chain CDR1 of SEQ ID NO: 37, light chain CDR2 of SEQ ID NO: 38, and light chain CDR3 of SEQ ID NO: 39,

M is a light chain constant domain of the antibody,

N is a heavy chain variable domain of the antibody that specifically binds to EGFR, the domain including heavy chain CDR1 of SEQ ID NO: 40, heavy chain CDR2 of SEQ ID NO: 41, and heavy chain CDR3 of SEQ ID NO: 42,

O is a CH1 domain in heavy chain constant domains of the antibody,

P is an Fc region or a fragment thereof, and

each of L6 and L7 is a peptide linker.

[Claim 13]

The third fusion protein of claim 12, wherein each of L6 and L7 is a peptide linker consisting of 1 to 50 amino acids.

[Claim 14]

The third fusion protein of claim 12, wherein L6 is a peptide linker consisting of the amino acid sequence of SEQ ID NO: 43.

[Claim 15]

The third fusion protein of claim 12, wherein L7 is a peptide linker consisting of the amino acid sequence of SEQ ID NO: 45.

[Claim 16]

The third fusion protein of claim 12, wherein the third fusion protein consists of the amino acid sequence of SEQ ID NO: 5.

[Claim 17]

A bispecific antibody, comprising:
the first fusion protein of claim 1; and
the second fusion protein of claim 7.

[Claim 18]

The bispecific antibody of claim 17, wherein a knob modification is included in I of the first fusion protein and a hole modification is included in J of the second fusion protein; or a hole modification is included in I of the first fusion protein and a knob modification is included in J of the second fusion protein.

[Claim 19]

A trispecific antibody, comprising:
the first fusion protein of claim 1; and
the third fusion protein of claim 12.

[Claim 20]

The trispecific antibody of claim 19, wherein a knob modification is included in I of the first fusion protein and a hole modification is included in P of the third fusion protein; or a hole modification is included in I of the first fusion protein and a knob modification is included in P of the third fusion protein.

[Claim 21]

A pharmaceutical composition for use in treating cancer, comprising as an active ingredient:

the first fusion protein of claim 1;
the second fusion protein of claim 7;
the third fusion protein of claim 12,
the bispecific antibody of claim 17; or

the trispecific antibody of claim 19.

[Claim 22]

Use of:

the first fusion protein of claim 1;

the second fusion protein of claim 7;

the third fusion protein of claim 12,

the bispecific antibody of claim 17; or

the trispecific antibody of claim 19

in the manufacture of a medicament for treating cancer.