

SUMMARY

TECHNICAL PROBLEM

[0016]

An object of the present disclosure is to provide a novel bispecific antibody that
5 binds to TfR and a cell surface antigen, a bispecific antibody fragment thereof, a DNA
encoding the bispecific antibody or the bispecific antibody fragment thereof, a vector
containing the DNA, a hybridoma and a transformant that produce the bispecific antibody or
the bispecific antibody fragment thereof, a method for producing the bispecific antibody or
the bispecific antibody fragment thereof, therapeutic and diagnostic agents containing the
10 bispecific antibody or the bispecific antibody fragment thereof, therapeutic and diagnostic
methods using the bispecific antibody or the bispecific antibody fragment thereof, and a
reagent for detection or measurement containing the bispecific antibody or the bispecific
antibody fragment thereof.

15 SOLUTION TO PROBLEM

[0017]

As means for solving the above problems, the present disclosure provides a
bispecific antibody or a bispecific antibody fragment thereof, which binds to TfR and a cell
surface antigen, and in which a polypeptide having an antigen-binding domain to a cell
20 surface antigen (also referred to as N-terminal side polypeptide) binds directly or through a
linker to the N-terminal side of an IgG portion that binds to TfR.

[0018]

That is, the present disclosure relates to the following.

1. A bispecific antibody or a bispecific antibody fragment thereof, which binds to a
25 transferrin receptor (TfR) and a cell surface antigen, including an IgG portion that binds to
TfR and an N-terminal side polypeptide that binds to the cell surface antigen, wherein the
polypeptide binds directly or through a linker to the N terminus of a heavy chain of the IgG
portion.

2. The bispecific antibody or the bispecific antibody fragment thereof according to
30 the above 1, wherein the IgG portion includes a heavy chain variable region (VH) having
complementarity determining region (CDR) 1 containing the amino acid sequence
represented by SEQ ID NO: 32 or an amino acid sequence modified from the amino acid

31. The use according to the above 30, wherein the disease associated with at least one of human TfR and a cell surface antigen is cancer.

32. A reagent for detecting or measuring at least one of human TfR and a cell surface antigen, containing the bispecific antibody or the bispecific antibody fragment thereof according to any one of the above 1 to 19.

[0018a]

In one aspect, the present disclosure refers to a bispecific antibody, which binds to a transferrin receptor (TfR) and a cell surface antigen, including an IgG portion that binds to TfR and an N-terminal side polypeptide that binds to the cell surface antigen, wherein the N-terminal side polypeptide binds directly or through a linker to the N terminus of a heavy chain of the IgG portion, wherein the IgG portion includes one VH selected from the following (ai) to (ci), and VL having CDRs 1 to 3 containing the amino acid sequences represented by SEQ ID NOS: 17 to 19, respectively: (ai) VH having CDRs 1 to 3 containing the amino acid sequences represented by SEQ ID NOS: 32 to 34, respectively; (bi) VH having CDRs 1 to 3 containing the amino acid sequences represented by SEQ ID NOS: 42 to 44, respectively; and (ci) VH having CDRs 1 to 3 containing the amino acid sequences represented by SEQ ID NOS: 47 to 49, respectively wherein the cell surface antigen is EGFR, and wherein the N-terminal side polypeptide is Fab of an antibody against EGFR, and the antibody is an antibody including VH having CDRs 1 to 3 containing the amino acid sequences represented by SEQ ID NOS: 60 to 62, respectively, or SEQ ID NOS: 65 to 67, respectively, and VL having CDRs 1 to 3 containing the amino acid sequences represented by SEQ ID NOS: 17 to 19, respectively.

[0018b]

In one aspect, the present disclosure refers to a bispecific antibody, which divalently binds to each of a transferrin receptor (TfR) and a cell surface antigen, including an IgG portion that binds to TfR and an N-terminal side polypeptide that binds to the cell surface antigen, wherein the N-terminal side polypeptide directly binds to the N terminus of a heavy chain of the IgG portion, wherein the IgG portion includes VH containing the amino acid sequence represented by SEQ ID NO: 31 and VL containing the amino acid sequence represented by SEQ ID NO: 16, wherein the cell surface antigen is EGFR, and wherein the N-terminal side polypeptide is Fab of an antibody against EGFR, and the antibody is an antibody including VH containing the amino acid sequence represented by SEQ ID NO: 64 and VL containing the amino acid sequence represented by SEQ ID NO: 16.

[0018c]

In one aspect, the present disclosure refers to a bispecific antibody, which divalently binds to each of a transferrin receptor (TfR) and a cell surface antigen, including an IgG portion that binds to TfR and an N-terminal side polypeptide that binds to the cell surface antigen, wherein the N-terminal side polypeptide directly binds to the N terminus of a heavy chain of the IgG portion, wherein the IgG portion includes VH containing the amino acid sequence represented by SEQ ID NO: 41 and VL containing the amino acid sequence represented by SEQ ID NO: 16, wherein the cell surface antigen is EGFR, and wherein the N-

terminal side polypeptide is Fab of an antibody against EGFR, and the antibody is an antibody including VH containing the amino acid sequence represented by SEQ ID NO: 59 and VL containing the amino acid sequence represented by SEQ ID NO: 16.

[0018d]

5 In one aspect, the present disclosure refers to a bispecific antibody, which divalently binds to each of a transferrin receptor (TfR) and a cell surface antigen, including an IgG portion that binds to TfR and an N-terminal side polypeptide that binds to the cell surface antigen, wherein the N-terminal side polypeptide directly binds to the N terminus of a heavy chain of the IgG portion, wherein the IgG portion includes VH containing the amino acid
10 sequence represented by SEQ ID NO: 41 and VL containing the amino acid sequence represented by SEQ ID NO: 16, wherein the cell surface antigen is EGFR, and wherein the N-terminal side polypeptide is Fab of an antibody against EGFR, and the antibody is an antibody including VH containing the amino acid sequence represented by SEQ ID NO: 64 and VL containing the amino acid sequence represented by SEQ ID NO: 16.

15 [0018e]

In one aspect, the present disclosure refers to a DNA encoding the bispecific antibody disclosed herein.

[0018f]

20 In one aspect, the present disclosure refers to a recombinant vector comprising the DNA disclosed herein.

[0018g]

In one aspect, the present disclosure refers to a transformant, obtainable by introducing the recombinant vector disclosed herein into a host cell.

[0018h]

25 In one aspect, the present disclosure refers to a method for producing the bispecific antibody disclosed herein, comprising culturing the transformant disclosed herein in a culture medium, allowing the transformant to produce and accumulate the bispecific antibody disclosed herein in a culture, and collecting the bispecific antibody from the culture.

[0018i]

30 In one aspect, the present disclosure refers to the bispecific antibody disclosed herein for use in therapy and/or diagnosis for a disease associated with at least one of human TfR and a cell surface antigen.

[0018j]

35 In one aspect, the present disclosure refers to use of the bispecific antibody disclosed herein in the manufacture of a medicament for treating a disease associated with at least one of human TfR and a cell surface antigen, wherein the disease associated with at least one of human TfR and a cell surface antigen is cancer.

[0018k]

40 In one aspect, the present disclosure refers to a reagent for detecting or measuring at least one of human TfR and a cell surface antigen, comprising the bispecific antibody disclosed herein.

ADVANTAGEOUS EFFECTS OF DISCLOSURE

[0019]

According to the present disclosure, a novel bispecific antibody that binds to TfR and a cell surface antigen, a bispecific antibody fragment thereof, a DNA encoding the bispecific antibody or the bispecific antibody fragment thereof, a vector containing the DNA, a hybridoma and a transformant that produce the bispecific antibody or the bispecific antibody fragment thereof, a method for producing the bispecific antibody or the bispecific antibody fragment thereof, therapeutic and diagnostic agents containing the bispecific antibody or the bispecific antibody fragment thereof, therapeutic and diagnostic methods using the bispecific antibody or the bispecific antibody fragment thereof, and a reagent for detection or measurement containing the bispecific antibody or the bispecific antibody fragment thereof can be provided.

BRIEF DESCRIPTION OF DRAWINGS

[0020]

[Fig. 1] Fig. 1 shows an example of a structure of a bispecific antibody of the present invention. Fig. 1(A) shows a bispecific antibody in which an N-terminal side polypeptide is Fab, and a polypeptide including VH of Fab is bound to the N terminus of an IgG portion. Fig. 1(B) shows a bispecific antibody in which an N-terminal side polypeptide is VHH. Fig. 1(C) shows a bispecific antibody in which an N-terminal side polypeptide is Fab, and a polypeptide including VL of Fab is bound to the N terminus of an IgG portion.

[Fig. 2] Fig. 2 shows the results of evaluating the expression level of an antigen in various types of cancer cell lines by flow cytometry. Figs. 2(A) to 2(C) show the results of evaluating the expression level of EGFR in OE21 cells, T.Tn cells, and U-937 cells, respectively, by a flow cytometer. Figs. 2(D) to 2(F) show the results of evaluating the expression level of TfR in OE21 cells, T.Tn cells, and U-937 cells, respectively, by a flow