[Problems to be Solved by the Invention]

The present invention was achieved in view of the above circumstances. An objective of the present invention is to provide polypeptide complexes that enable cancer treatment by having T cells close to target cancer cells and using the cytotoxicity of T cells against the target cancer cells, methods for producing the polypeptide complexes, and therapeutic agents containing such a polypeptide complex as an active ingredient for inducing cellular cytotoxicity. Another objective of the present invention is to provide pharmaceutical compositions for treating or preventing various cancers, which comprise an above-mentioned therapeutic agent for inducing cellular cytotoxicity as an active ingredient, and therapeutic methods using the pharmaceutical compositions.

[Means for Solving the Problems]

The present inventors discovered novel polypeptide complexes that retain the strong anti-tumor activity possessed by BiTE and have a long half-life in blood, as well as excellent safety properties that result in no induction of cancer antigen-independent cytokine storm or such. The present inventors also found that the polypeptide complexes can damage various target cells when antigen-binding domains of the polypeptide complexes are substituted. Based on the above findings, the present inventors demonstrated that the polypeptide complexes of the present invention damage cancer cells. The present inventors also revealed that more efficient cellular cytotoxicity is achieved by regulating the CH1/CL interface association and introducing Knobs-into-Holes (KiH) modifications into the polypeptide complexes. In addition, the present inventors demonstrated that various cancers can be treated or prevented using therapeutic agents for inducing cellular cytotoxicity comprising a polypeptide complex of the present invention as an active ingredient.

Thus, in a first aspect the present invention refers to a polypeptide complex which comprises:

(1) an antigen-binding domain;

(2) a domain comprising an Fc region that has reduced Fcγ receptor-binding activity; and

(3) a CD3-binding domain, wherein the antigen-binding domain and CD3-binding domain are each a monovalent Fab,

(i) in which the heavy chain Fv fragment of a monovalent Fab forming the antigen-binding domain is linked to one of the polypeptides forming the Fc domain *via* a CH1 domain and the light chain Fv fragment of the Fab is linked to a CL domain; and the light chain Fv fragment of Fab forming the CD3-binding domain is linked to the other polypeptide forming the Fc domain *via* a CH1 domain and the heavy chain Fv fragment of the Fab is linked to a CL domain;

(ii) in which the heavy chain Fv fragment of a monovalent Fab forming the antigen-binding domain is linked to one of the polypeptides forming the Fc domain *via* a CH1 domain; the light chain Fv fragment of the Fab is linked to a CL domain; the heavy chain Fv fragment of the Fab forming the CD3-binding domain is linked to the other polypeptide forming the Fc domain *via* a CL domain; and the light chain Fv fragment of the Fab is linked to a CH1 domain;

(iii) in which the heavy chain Fv fragment of a monovalent Fab forming the CD3-binding domain is linked to one of the polypeptides forming the Fc domain *via* a CH1 domain; the light chain Fv fragment of the Fab is linked to a CL domain; the light chain Fv fragment of Fab forming the antigen-binding domain is linked to the other polypeptide forming the Fc domain *via* a CH1 domain; and the heavy chain Fv fragment of the Fab is linked to a CL domain; or

(iv) in which the heavy chain Fv fragment of a monovalent Fab forming the CD3-binding domain is linked to one of the polypeptide forming the Fc domain *via* a CH1 domain; the light chain Fv fragment of the Fab is linked to a CL domain; the heavy chain Fv fragment of the Fab forming the antigen-binding domain to the other polypeptide forming the Fc domain *via* a CL domain; and the light chain Fv fragment of the Fab is linked to a CH1 domain.

In a second aspect the present invention refers to a polynucleotide encoding the polypeptide complex of the present invention, a vector comprising the said polynucleotide, or a cell comprising the said vector.

In a third aspect the present invention refers to a method for producing a polypeptide complex, which comprises culturing the cell of the present invention and isolating the polypeptide complex from the culture supernatant.

In a fourth aspect the present invention refers to a therapeutic agent for cancer, which comprises as an active ingredient the polypeptide complex of the present invention.

More specifically, the present disclosure also describes~~invention provides~~ the following:

[1] a polypeptide complex which comprises:

(1) an antigen-binding domain;

(2) a domain comprising an Fc region that has reduced Fcγ receptor-binding activity; and

(3) a T cell receptor complex-binding domain;

[2] the polypeptide complex of [1], wherein the T cell receptor complex-binding domain is a T cell receptor-binding domain;

[3] the polypeptide complex of [1], wherein the T cell receptor complex-binding domain is a CD3-binding domain;

[4] the polypeptide complex of any one of [1] to [3], wherein the antigen-binding domain is a bivalent antigen-binding domain;

[5] the polypeptide complex of [4], wherein the bivalent antigen-binding domain is a domain

cancer.

The present disclosureinvention also describesrelates to kits for use in a method as described hereinof the present invention, which contain a polypeptide complex as described hereinof the present invention or a polypeptide complex produced by a method as described hereinof the present invention. The present disclosureinvention also describesrelates to use of a polypeptide complex as described hereinof the present invention or a polypeptide complex produced by a method as described hereinof the present invention in producing a therapeutic agent for inducing cellular cytotoxicity. The present disclosureinvention also describesrelates to polypeptide complexes as described hereinof the present invention or polypeptide complexes produced by methods as described hereinof the present invention for use in a method as described hereinof the present invention.

[Effects of the Invention]

The present invention provides novel polypeptide complexes that retain the strong anti-tumor activity of BiTE and have a long half-life in blood, as well as excellent safety properties that result in no induction of cancer antigen-independent cytokine storm or such. When the antigen-binding domain of a polypeptide complex of the present invention is substituted, therapeutic agents that comprise the polypeptide complex as an active ingredient for inducing cellular cytotoxicity can target and damage various cells including cancer cells. Thus, various cancers can be treated or prevented. This allows desirable treatments that are highly safe and convenient, and reduce the physical burden for patients.

Brief Description of the Drawings

Fig. 1 is a graph showing comparison of the cytotoxic activities of GPC3 ERY1 (GPC3 BiTE), GPC3 ERY2, and an IgG-type anti-GPC3 antibody. Closed square (■), closed triangle (▲), and open square (□) represent the cytotoxic activities of GPC3 ERY1 (GPC3 BiTE), GPC3 ERY2, the IgG-type anti-GPC3 antibody, respectively.

Fig. 2 is a graph showing comparison of the cytotoxic activities of GPC3 BiTE and GPC3 ERY5. Closed square (■) and open circle (○) represent the cytotoxic activities of GPC3 BiTE and GPC3 ERY5, respectively.

Fig. 3 is a graph showing comparison of the cytotoxic activities of GPC3 BiTE and GPC3 ERY6. Closed square (■) and closed triangle (▲) represent the cytotoxic activities of GPC3 BiTE and GPC3 ERY6, respectively.

Fig. 4 is a graph showing comparison of the cytotoxic activities of GPC3 BiTE and GPC3 ERY7. Closed square (■) and closed diamond (♦) represent the cytotoxic activities of GPC3 BiTE and