CLAIMS

1. A method of producing a library, the method comprising the steps of:

(a) identifying amino acid sites that fulfill any one or more of (i) to (iii) below in antigen-binding domains whose antigen-binding activity varies depending on the concentration of a small molecule compound or in antigen-binding domains that have binding activity to a small molecule compound:

(i) one or more amino acid sites that are not involved in the binding to the small molecule compound;

(ii) one or more amino acid sites that show diversity of amino acid occurrence frequency in the antibody repertoire of the animal species to which the parent antigen-binding domain belongs; and

(iii) one or more amino acid sites that are not important for canonical structure formation; and

(b) designing and producing a library that comprises nucleic acids encoding unmodified antigen-binding domains/molecules, and nucleic acids that encode individually a plurality of variants of the aforementioned antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain which have different sequences from one another and have modifications at one or more of the amino acid sites identified in step (a); and(c) contacting the library of step (b) comprising a plurality of antigen-binding molecules having binding activity to a small molecule compound with the small molecule compound; and

(d) concentrating nucleic acids from the library, wherein the nucleic acids encode a plurality of variants of antigen-binding molecules having binding activity to the small molecule compound;

wherein the produced library comprises mainly:

(i) a plurality of antigen-binding molecules comprising an antigen-binding domain, which have different sequences from one another; or

(ii) nucleic acids that encode the plurality of antigen-binding molecules comprising an antigen-binding domain, which have different sequences from one another;

wherein the aforementioned antigen-binding domains are antigen-binding domains whose antigen-binding activity varies depending on the concentration of a small molecule compound; and wherein the antigen-binding domains comprise an antibody heavy-chain variable region and an antibody light-chain variable region; and wherein the small molecule compound is a cancer tissue-specific compound and is at least one compound selected from the group consisting of nucleosides that have a purine ring structure, amino acids and their metabolites, lipids and their metabolites, primary metabolites from sugar metabolism, and nicotinamide and its metabolites.

2. A method of producing a library, the method comprising the steps of:

(a) identifying amino acid sites that fulfill any one or more of (i) to (iii) below in antigen-binding domains whose antigen-binding activity varies depending on the concentration of a small molecule compound or in antigen-binding domains that have binding activity to a small molecule compound:

(i) one or more amino acid sites that are not involved in the binding to the small molecule compound;

(ii) one or more amino acid sites that show diversity of amino acid occurrence frequency in the antibody repertoire of the animal species to which the parent antigen-binding domain belongs; and

(iii) one or more amino acid sites that are not important for canonical structure formation;

(b) producing a plurality of variants of the aforementioned antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain, which have different sequences from one another and have modifications at one or more of the amino acid sites identified in step (a);

(c) identifying one or more amino acid modifications that do not substantially change the binding activity of each of the aforementioned variants to the small molecule compound; and

(d) producing a library comprising nucleic acids that encode unmodified antigen-binding domains/molecules, and nucleic acids that encode a plurality of variants of the aforementioned antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain, which have different sequences from one another and have one or more of the amino acid modifications identified in step (c); and

(e) contacting the library of step (d) comprising a plurality of antigen-binding molecules having binding activity to a small molecule compound with the small molecule compound; and

(f) concentrating nucleic acids from the library, wherein the nucleic acids encode a plurality of variants of antigen-binding molecules having binding activity to the small molecule compound;

wherein the produced library comprises mainly:

(i) a plurality of antigen-binding molecules comprising an antigen-binding domain, which have different sequences from one another; or

(ii) nucleic acids that encode the plurality of antigen-binding molecules comprising an antigen-binding domain, which have different sequences from one another;

wherein the aforementioned antigen-binding domains are antigen-binding domains whose antigen-binding activity varies depending on the concentration of a small molecule compound; and wherein the antigen-binding domains comprise an antibody heavy-chain

variable region and an antibody light-chain variable region; and wherein the small molecule compound is a target tissue-specific compound and is at least one compound selected from the group consisting of nucleosides that have a purine ring structure, amino acids and their metabolites, lipids and their metabolites, primary metabolites from sugar metabolism, and nicotinamide and its metabolites.

3. A method for producing an antigen-binding molecule comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound, which comprises the steps of:

(a) contacting a library with an antigen in the absence of a small molecule compound;

(b) selecting an antigen-binding domain that does not bind to the antigen in step (a) above;

(c) contacting the antigen-binding domain selected in step (b) above with the antigen in the presence of the small molecule compound;

(d) selecting the antigen-binding domain that binds to the antigen in step (c) above;

(e) linking the polynucleotide that encodes the antigen-binding domain selected in step (d) above with a polynucleotide that encodes a polypeptide comprising an Fc region;

(f) culturing a cell introduced with a vector in which the polynucleotide obtained in step (e) above is operably linked; and

(g) collecting the antigen-binding molecule from the culture solution of the cell cultured in step (f) above,

wherein the library used in step (a) is produced by the method comprising the steps of:

(i) contacting a library comprising a plurality of antigen-binding molecules having binding activity to a small molecule compound with the small molecule compound; and

(ii) concentrating nucleic acids from the library, wherein the nucleic acids encode a plurality of variants of antigen-binding molecules having binding activity to the small molecule compound,

wherein the produced library comprises mainly:

(i) a plurality of antigen-binding molecules comprising an antigen-binding domain, which have different sequences from one another; or

(ii) nucleic acids that encode the plurality of antigen-binding molecules comprising an antigen-binding domain, which have different sequences from one another,

wherein the antigen-binding domain comprises an antibody heavy chain variable region and an antibody light chain variable region,

wherein the small molecule compound is a cancer tissue specific compound and is at least one compound selected from the group consisting of nucleosides that have a purine ring structure, amino acids and their metabolites, lipids and their metabolites, primary metabolites from sugar metabolism, and nicotinamide and its metabolites.

4. A method for producing an antigen-binding molecule comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound, which comprises the steps of:

(a) contacting a library with an antigen in the presence of a small molecule compound;

(b) collecting an antigen-binding domain by dissociating it using the small molecule compound at a lower concentration than in step (a) above;

(c) linking the polynucleotide that encodes the antigen-binding domain collected in step (b) above with a polynucleotide that encodes a polypeptide comprising an Fc region;

(d) culturing a cell introduced with a vector in which the polynucleotide obtained in step (c) above is operably linked; and

(e) collecting the antigen-binding molecule from the culture solution of the cell cultured in step (d) above,

wherein the library used in step (a) is produced by the method comprising the steps of:

(i) contacting a library comprising a plurality of antigen-binding molecules having binding activity to a small molecule compound with the small molecule compound; and

(ii) concentrating nucleic acids from the library, wherein the nucleic acids encode a plurality of variants of antigen-binding molecules having binding activity to the small molecule compound,

wherein the produced library comprises mainly:

(i) a plurality of antigen-binding molecules comprising an antigen-binding domain, which have different sequences from one another; or

(ii) nucleic acids that encode the plurality of antigen-binding molecules comprising an antigen-binding domain, which have different sequences from one another,

wherein the antigen-binding domain comprises an antibody heavy chain variable region and an antibody light chain variable region,

wherein the small molecule compound is a cancer tissue specific compound and is at least one compound selected from the group consisting of nucleosides that have a purine ring structure, amino acids and their metabolites, lipids and their metabolites, primary metabolites from sugar metabolism, and nicotinamide and its metabolites.

5. The method of any one of claims 1 to 4 is produced by a method comprising any one of the steps of:

i) designing a library by concentrating nucleic acids that encode a plurality of variants of antigen-binding molecules having binding activity to a small molecule compound from a library which comprises nucleic acids encoding one or more variants produced by modifying amino acids positioned in the heavy chain variable regions;

ii) designing a library by concentrating nucleic acids that encode a plurality of variants of antigen-binding molecules having binding activity to a small molecule compound from a library which comprises nucleic acids encoding one or more variants produced by modifying amino acids positioned in the light chain variable regions; and

iii) designing a library by combining the antigen-binding molecule-encoding nucleic acids concentrated from each of the variable region libraries of steps i) and ii).

6. The method of any one of claims 1 to 5, wherein the aforementioned antigen-binding molecules are fusion polypeptides formed by fusing an antigen-binding domain with at least a portion of a virus coat protein.

7. The method of any one of claims 1 to 5, wherein the aforementioned antigen-binding molecules are antigen-binding molecules comprising antibody heavy chains and light chains, and the method further comprises a step of designing a synthetic library of the heavy chains and/or light chains.

8. The method of claim 7, wherein the antibody heavy chain variable regions and/or light chain variable regions comprise a germline-derived framework sequence.

9. The method of any one of the claims 1 to 8, wherein the small molecule compound is adenosine, adenosine monophosphate, adenosine diphosphate, adenosine triphosphate or kynurenine.

10. The method of any one of claims 1, 2 and 5 to 11, wherein the amino acid sites not involved in binding with the small molecule compound sites are sites other than any one or more of the amino acids selected from below:
H chain: 33, 50, 52, 56, 57, 58, 99, 100, 100a, 54, 55, 97, 100c, 101, 94, 95, 100d, 100e (Kabat Numbering); and

L chain: 95c, 96, 95a, 95b, 49, 55 (Kabat Numbering).