objectives. As a result, they generated antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of the target tissue-specific compound. Furthermore, the present inventors discovered that the antigen-binding molecules or pharmaceutical compositions comprising the antigen-binding molecules are useful for treating diseases that originate from a target tissue, and that they are also useful for treatment of diseases originating from target tissues that includes administering the antigen-binding molecules. They also discovered that the antigen-binding molecules are useful in the production of pharmaceuticals for treating diseases that originate from target tissues.

The present inventors also successfully produced a library comprising a plurality of antigen-binding molecules having different sequences from one another, wherein the molecules have an antigen-binding domain that comprises amino acid residues involved in binding with a small molecule that may cause the antigen-binding activity of the antigen-binding molecule to vary according to differences in the *in vivo* environmental factors or depending on administration of an unnatural compound. They also created methods for screening and producing the antigen-binding molecules using the library, and thereby completed the present invention.

The present invention is based on such findings, and specifically includes embodiments exemplified below.

[Embodiment 1]

A library that comprises mainly:

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

wherein the aforementioned antigen-binding molecule domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound.

[Embodiment 2]

The library of Embodiment [1], which is produced by a 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;

More preferably, the library of the present invention can be produced by increasing the ratio of presence of nucleic acids encoding variants having the desired activity by 1.2 times or more, 1.5 times or more, 2 times or more, 4 times or more, 10 times or more, 25 times or more, or 100 times or more.

Library production method

The invention of the present application also relates to methods for producing various embodiments of "libraries" included in the invention of this application described above.

The "library production method" of the invention of the present application is not limited to any of the specific methods shown as examples below, and includes any method that can produce the above-described "libraries" of the invention of the present application.

For example, "library production method" in the invention of the present application include the methods shown as examples below.

Each of the specific matters in the "library production method" shown as examples below has technical significance as described in detail above with regard to the "library" in the invention of the present application.

(Example 1)

A method for producing a library that comprises mainly

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

wherein the aforementioned antigen-binding molecule domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound;

wherein the method comprises the steps of (a) and (b) below:

(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 a library comprising nucleic acids that encode 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).

(Example 2)

A method for producing a library that comprises mainly

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

wherein the aforementioned antigen-binding molecule domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound;

wherein the method comprises the steps of (a) to (d) below:

(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).

(Example 3)

A method for producing a library that comprises mainly

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

wherein the aforementioned antigen-binding molecule domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound;

wherein the method comprises the steps of 1) and 2) below:

1) contacting a library that comprises a plurality of antigen-binding molecules having binding activity to a small molecule compound with the small molecule compound; and

2) concentrating from the library, nucleic acids that encode a plurality of variants of antigen-binding molecules having binding activity to the small molecule compound.

(Example 4)

A method for producing a library that comprises mainly

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

wherein the aforementioned antigen-binding molecule domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound;

wherein the method comprises any one of steps 1) to 3) below:

1) 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 the library of (Example 3) which comprises nucleic acids encoding one or more variants produced

The library production method of any one of (Example 1) to (Example 11) described above, wherein the amino acid sites not involved in binding with the small molecule compound are sites other than any one or more of the amino acids selected from below:

H chain: 97, 100c, 101, 94, 95, 100d, 100e, 33, 50, 52, 56, 57, 58, 99, 100, 100a, 54, 55 (Kabat Numbering); and

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

Library (other embodiments)

An embodiment of a library of the present invention that can yield antigen-binding domains whose antigen-binding ability varies depending on the concentration of a small molecule compound is, for example, the library below.

A library that comprises mainly:

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

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

wherein the antigen-binding molecule domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain having binding activity to a small molecule compound. A library of this embodiment preferably has diversity of 1.2×10^8 or higher.

The term "comprises mainly" in the description of a library that comprises mainly a plurality of antigen-binding molecules in this embodiment reflects the number of antigen-binding molecules having binding activity to a small molecule compound (for example, a target tissue-specific compound) among the number of independent clones that differ in sequence in the library. Specifically, presence of at least 10^4 antigen-binding molecules that exhibit such binding activity in the library is preferred. In other words, the term may be suitably expressed as the ratio of antigen-binding molecules in which the antigen-binding activity of the antigen-binding domain differs depending on the presence or absence of the small molecule to the number of the independent clones that differ in sequence in the library. Specifically, the antigen-binding domains of the present invention can be obtained from a library that comprises antigen-binding molecules that exhibit such binding activity at a ratio of 10^{-6}% to 80%, or 10^{-5}% to 60%, preferably 10^{-4}% to 40%, more preferably 10^{-3}% to 40%, and even more preferably 10^{-2}% to 40% to the number of the independent clones that differ in sequence in the library. Similar to the case above, fusion polypeptides, polynucleotide molecules, or vectors can also be presented as the number of molecules or the ratio to all molecules. In addition, viruses can also

A library that comprises mainly:

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

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

wherein the antigen-binding molecule domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain that have binding activity to a small molecule compound, and the library is for obtaining antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound.

The term "comprises mainly" in the description of a library that comprises mainly a plurality of antigen-binding molecules in this embodiment reflects the number of antigen-binding molecules having binding activity to a small molecule compound (for example, a target tissue-specific compound) among the number of independent clones that differ in sequence in the library. Specifically, presence of at least 10^4 antigen-binding molecules that exhibit such binding activity in the library is preferred. In other words, the term may be suitably expressed as the ratio of antigen-binding molecules in which the antigen-binding activity of the antigen-binding domain differs depending on the presence or absence of the small molecule to the number of independent clones that differ in sequence in the library. Specifically, the antigen-binding domains of the present invention can be obtained from a library comprising antigen-binding molecules that exhibit such binding activity at a ratio of 10^{-6}% to 80%, or 10^{-5}% to 60%, preferably 10^{-4}% to 40%, more preferably 10^{-3}% to 40%, and even more preferably 10^{-2}% to 40% to the number of independent clones that differ in sequence in the library. As in the above case, fusion polypeptides, polynucleotide molecules, or vectors can also be presented as the number of molecules or the ratio to all molecules. In addition, viruses can also be presented as the number of individual viruses or the ratio to all viruses as in the above case.

<u>Amino acids that change the antigen-binding activity of an antigen-binding domain depending on the presence or absence of a small molecule</u>

With respect to methods for obtaining (methods of screening for) templates (antigen-binding domains whose antigen-binding activity varies depending on the concentration of a small molecule compound, or antigen-binding domains having binding activity to a small molecule compound) to be used when producing a library of the present invention, these antigen-binding domains and such may be prepared in any manner. It is possible to use pre-existing antigen-binding domains or antibodies, and pre-existing libraries (phage libraries,

CLAIMS

1. A library that comprises mainly:

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

wherein the aforementioned antigen-binding molecule domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound.

2. The library of claim 1, which is produced by a 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 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).

3. The library of claim 2 which is produced by a 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).

4. The library of claim 1 produced by a method comprising the steps of:

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

2) concentrating from the library, nucleic acids that encode a plurality of variants of antigen-binding molecules having binding activity to the small molecule compound.

5. The library of claim 4, wherein the aforementioned antigen-binding molecules are antigen-binding molecules that comprise heavy-chain variable regions and light-chain variable regions of an antibody, and wherein the library is produced by a method comprising any one of the steps of:

1) 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 the library of claim 4 which comprises nucleic acids encoding one or more variants produced by modifying amino acids positioned in the heavy chain variable regions;

2) 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 the library of claim 4 which comprises nucleic acids encoding one or more variants produced by modifying amino acids positioned in the light chain variable regions; and

3) designing a library by combining the antigen-binding molecule-encoding nucleic acids concentrated from each of the variable region libraries of steps 1) and 2).

6. The library 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 library 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 library further comprises a step of designing a synthetic library of the heavy chains and/or

light chains.

8. The library of claim 7, wherein the antibody heavy chains and/or light chains comprise a germline-derived framework sequence.

9. The library of any one of claims 1 to 8, wherein the aforementioned small molecule compound is a target tissue-specific compound or an unnatural compound.

10. The library of any one of claims 1 to 9, wherein the aforementioned target tissue is a cancer tissue or an inflammatory tissue.

11. The library of claim 10, wherein the cancer tissue-specific compound 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.

12. The library of any one of claims 1 to 11, wherein the small molecule compound is kynurenine, adenosine, adenosine monophosphate, adenosine diphosphate, or adenosine triphosphate.

13. The library of any one of claims 1 to 12, wherein the amino acid sites not involved in binding with the small molecule compound are sites other than any one or more of the amino acids selected from below:

H chain: 97, 100c, 101, 94, 95, 100d, 100e, 33, 50, 52, 56, 57, 58, 99, 100, 100a, 54, 55 (Kabat Numbering); and

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

14. 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 the library of any one of claims 1 to 13 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.

15. 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 the library of any one of claims 1 to 13 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.

16. The method of claim 14 or 15 for producing an antigen-binding molecule that comprises an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound, which further comprises the steps of:

(a) contacting the library of any one of claims 1 to 13 with a small molecule compound; and

(b) selecting antigen-binding domains collected in step (a) above.

17. The method of any one of claims 14 to 16 for producing an antigen-binding molecule, wherein the small molecule compound is kynurenine, adenosine, adenosine monophosphate, adenosine diphosphate, or adenosine triphosphate.

18. An antigen-binding molecule comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of an unnatural compound.

19. A pharmaceutical composition that comprises the antigen-binding molecule of claim 18.

objectives. As a result, they generated antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of the target tissue-specific compound. Furthermore, the present inventors discovered that the antigen-binding molecules or pharmaceutical compositions comprising the antigen-binding molecules are useful for treating diseases that originate from a target tissue, and that they are also useful for treatment of diseases originating from target tissues that includes administering the antigen-binding molecules. They also discovered that the antigen-binding molecules are useful in the production of pharmaceuticals for treating diseases that originate from target tissues.

The present inventors also successfully produced a library comprising a plurality of antigen-binding molecules having different sequences from one another, wherein the molecules have an antigen-binding domain that comprises amino acid residues involved in binding with a small molecule that may cause the antigen-binding activity of the antigen-binding molecule to vary according to differences in the *in vivo* environmental factors or depending on administration of an unnatural compound. They also created methods for screening and producing the antigen-binding molecules using the library, and thereby completed the present invention.

The present invention is based on such findings, and specifically includes embodiments exemplified below.

[Embodiment 1]

A library that comprises mainly:

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

wherein the aforementioned antigen-binding domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound.

[Embodiment 2]

The library of Embodiment [1], which is produced by a 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;

More preferably, the library of the present invention can be produced by increasing the ratio of presence of nucleic acids encoding variants having the desired activity by 1.2 times or more, 1.5 times or more, 2 times or more, 4 times or more, 10 times or more, 25 times or more, or 100 times or more.

Library production method

The invention of the present application also relates to methods for producing various embodiments of "libraries" included in the invention of this application described above.

The "library production method" of the invention of the present application is not limited to any of the specific methods shown as examples below, and includes any method that can produce the above-described "libraries" of the invention of the present application.

For example, "library production method" in the invention of the present application include the methods shown as examples below.

Each of the specific matters in the "library production method" shown as examples below has technical significance as described in detail above with regard to the "library" in the invention of the present application.

(Example 1)

A method for producing a library that comprises mainly

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

wherein the aforementioned antigen-binding domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound;

wherein the method comprises the steps of (a) and (b) below:

(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 a library comprising nucleic acids that encode 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).

(Example 2)

A method for producing a library that comprises mainly

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

wherein the aforementioned antigen-binding domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound;

wherein the method comprises the steps of (a) to (d) below:

(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).

(Example 3)

A method for producing a library that comprises mainly

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

wherein the aforementioned antigen-binding domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound;

wherein the method comprises the steps of 1) and 2) below:

1) contacting a library that comprises a plurality of antigen-binding molecules having binding activity to a small molecule compound with the small molecule compound; and

2) concentrating from the library, nucleic acids that encode a plurality of variants of antigen-binding molecules having binding activity to the small molecule compound.

(Example 4)

A method for producing a library that comprises mainly

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

wherein the aforementioned antigen-binding domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound;

wherein the method comprises any one of steps 1) to 3) below:

1) 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 the library of (Example 3) which comprises nucleic acids encoding one or more variants produced

The library production method of any one of (Example 1) to (Example 11) described above, wherein the amino acid sites not involved in binding with the small molecule compound are sites other than any one or more of the amino acids selected from below:

H chain: 97, 100c, 101, 94, 95, 100d, 100e, 33, 50, 52, 56, 57, 58, 99, 100, 100a, 54, 55 (Kabat Numbering); and

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

Library (other embodiments)

An embodiment of a library of the present invention that can yield antigen-binding domains whose antigen-binding ability varies depending on the concentration of a small molecule compound is, for example, the library below.

A library that comprises mainly:

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

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

wherein the antigen-binding domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain having binding activity to a small molecule compound. A library of this embodiment preferably has diversity of 1.2×10^8 or higher.

The term "comprises mainly" in the description of a library that comprises mainly a plurality of antigen-binding molecules in this embodiment reflects the number of antigen-binding molecules having binding activity to a small molecule compound (for example, a target tissue-specific compound) among the number of independent clones that differ in sequence in the library. Specifically, presence of at least 10^4 antigen-binding molecules that exhibit such binding activity in the library is preferred. In other words, the term may be suitably expressed as the ratio of antigen-binding molecules in which the antigen-binding activity of the antigen-binding domain differs depending on the presence or absence of the small molecule to the number of the independent clones that differ in sequence in the library. Specifically, the antigen-binding domains of the present invention can be obtained from a library that comprises antigen-binding molecules that exhibit such binding activity at a ratio of 10^{-6}% to 80%, or 10^{-5}% to 60%, preferably 10^{-4}% to 40%, more preferably 10^{-3}% to 40%, and even more preferably 10^{-2}% to 40% to the number of the independent clones that differ in sequence in the library.

Similar to the case above, fusion polypeptides, polynucleotide molecules, or vectors can also be presented as the number of molecules or the ratio to all molecules. In addition, viruses can also

A library that comprises mainly:

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

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

wherein the antigen-binding domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain that have binding activity to a small molecule compound, and the library is for obtaining antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound.

The term "comprises mainly" in the description of a library that comprises mainly a plurality of antigen-binding molecules in this embodiment reflects the number of antigen-binding molecules having binding activity to a small molecule compound (for example, a target tissue-specific compound) among the number of independent clones that differ in sequence in the library. Specifically, presence of at least 10^4 antigen-binding molecules that exhibit such binding activity in the library is preferred. In other words, the term may be suitably expressed as the ratio of antigen-binding molecules in which the antigen-binding activity of the antigen-binding domain differs depending on the presence or absence of the small molecule to the number of independent clones that differ in sequence in the library. Specifically, the antigen-binding domains of the present invention can be obtained from a library comprising antigen-binding molecules that exhibit such binding activity at a ratio of 10^{-6}% to 80%, or 10^{-5}% to 60%, preferably 10^{-4}% to 40%, more preferably 10^{-3}% to 40%, and even more preferably 10^{-2}% to 40% to the number of independent clones that differ in sequence in the library. As in the above case, fusion polypeptides, polynucleotide molecules, or vectors can also be presented as the number of molecules or the ratio to all molecules. In addition, viruses can also be presented as the number of individual viruses or the ratio to all viruses as in the above case.

Amino acids that change the antigen-binding activity of an antigen-binding domain depending on the presence or absence of a small molecule

With respect to methods for obtaining (methods of screening for) templates (antigen-binding domains whose antigen-binding activity varies depending on the concentration of a small molecule compound, or antigen-binding domains having binding activity to a small molecule compound) to be used when producing a library of the present invention, these antigen-binding domains and such may be prepared in any manner. It is possible to use pre-existing antigen-binding domains or antibodies, and pre-existing libraries (phage libraries,

CLAIMS

1. A library that comprises mainly:
(i) a plurality of antigen-binding domains or antigen-binding molecules comprising an antigenbinding domain, which have different sequences from one another; or
(ii) nucleic acids that encode the plurality of antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain, which have different sequences from one another;
wherein the aforementioned antigen-binding domains or antigen-binding molecules are antigen-binding domains or antigen-binding molecules comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound.

2. The library of claim 1, which is produced by a method comprising the steps of:
(a) identifying amino acid sites that fulfill any one or more of (i) to (iii) below in antigenbinding 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 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 antigenbinding domain which have different sequences from one another and have modifications at one or more of the amino acid sites identified in step (a).

3. The library of claim 2 which is produced by a 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).

4. The library of claim 1 produced by a method comprising the steps of:
1) contacting a library comprising a plurality of antigen-binding molecules having binding activity to a small molecule compound with the small molecule compound; and
2) concentrating from the library, nucleic acids that encode a plurality of variants of antigen-binding molecules having binding activity to the small molecule compound.

5. The library of claim 4, wherein the aforementioned antigen-binding molecules are antigen-binding molecules that comprise heavy-chain variable regions and light-chain variable regions of an antibody, and wherein the library is produced by a method comprising any one of the steps of:
1) 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 the library of claim 4 which comprises nucleic acids encoding one or more variants produced by modifying amino acids positioned in the heavy chain variable regions;
2) 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 the library of claim 4 which comprises nucleic acids encoding one or more variants produced by modifying amino acids positioned in the light chain variable regions; and
3) designing a library by combining the antigen-binding molecule-encoding nucleic acids concentrated from each of the variable region libraries of steps 1) and 2).

6. The library 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 library 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 library further comprises a step of designing a synthetic library of the heavy chains and/or light chains.

8. The library of claim 7, wherein the antibody heavy chains and/or light chains comprise a germline-derived framework sequence.

9. The library of any one of claims 1 to 8, wherein the aforementioned small molecule compound is a target tissue-specific compound or an unnatural compound.

10. The library of any one of claims 1 to 9, wherein the aforementioned target tissue is a cancer tissue or an inflammatory tissue.

11. The library of claim 10, wherein the cancer tissue-specific compound 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.

12. The library of any one of claims 1 to 11, wherein the small molecule compound is adenosine, adenosine monophosphate, adenosine diphosphate, adenosine triphosphate or kynurenine.

13. The library of any one of claims 1 to 12, wherein the amino acid sites not involved in binding with the small molecule compound 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).

14. 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 the library of any one of claims 1 to 13 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 35 (f) above.

15. 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 the library of any one of claims 1 to 13 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.

16. The method of claim 14 or 15 for producing an antigen-binding molecule that comprises an antigen-binding domain whose antigen-binding activity varies depending on the concentration of a small molecule compound, which further comprises the steps of:
(a) contacting the library of any one of claims 1 to 13 with a small molecule compound; and
(b) selecting antigen-binding domains collected in step (a) above.

17. The method of any one of claims 14 to 16 for producing an antigen-binding molecule, wherein the small molecule compound is adenosine, adenosine monophosphate, adenosine diphosphate, adenosine triphosphate or kynurenine.

18. An antigen-binding molecule comprising an antigen-binding domain whose antigen-binding activity varies depending on the concentration of an unnatural compound.

19. A pharmaceutical composition that comprises the antigen-binding molecule of claim 18.