[0007]

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Summary

[0008]

The present disclosure relates to peptides that can induce cytotoxic T cells (CTLs) specifically responding to CDCA1-expressing cells. When these peptides are presented on antigen-presenting cells (APCs) by the human leukocyte antigen (HLA) and recognized by CD8-positive T cells, CTLs that show a peptide-specific cytotoxic activities are induced. CDCA1-derived peptides that have been identified so far to have CTL-inducing ability (CTL inducibility) are HLA-A02-restricted, HLA-A24-restricted, HLA-A11-restricted, HLA-A33-restricted, or HLA-A03-restricted peptides, and when antigen-presenting cells do not express these HLAs, the peptides cannot induce CTLs. Therefore, conventional peptides are not suitable for performing immunotherapy on cancer patients (subjects) that do not have these HLAs. HLA-A01 is an HLA allele highly frequently observed in Caucasians (Cao K *et al.*, Hum Immunol 2001, 62(9): 1009-30). It is desirable to administer HLA-A01-restricted peptides to HLA-A01-positive cancer patients. Hence, the present disclosure relates to CDCA1-derived peptides with CTL-inducing ability that are restrictive to HLA-A01. Based on results disclosed herein, the peptides of the present disclosure have been proven to be epitope peptides that can induce a potent and specific immune response against cancer cells expressing CDCA1 and HLA-A01.

[0009]

Therefore, one of the objectives of the present disclosure is to provide CDCA1-derived peptides that can induce CTLs in an HLA-A01-restrictive manner. These peptides can be used to induce CTLs *in vitro*, *ex vivo* or *in vivo*, or can be used to administer to subjects for the purpose of inducing an immune response against CDCA1-expressing cancer cells. Preferable peptides are peptides comprising the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37; more preferable peptides are nonapeptides or decapeptides; and even more preferable peptides are peptides consisting of the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37.

[0010]

In one aspect, the present disclosure refers to a peptide of less than 15 amino acids having cytotoxic T cell (CTL)-inducing ability, which comprises the amino acid sequence selected from the group below: (a) the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37; and (b) the amino acid sequence in which one, two, three or several amino acids are substituted, deleted, inserted and/or added to the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37. In another aspect, the present disclosure refers to a polynucleotide, which encodes the peptide as described herein. In another aspect, the present disclosure refers to a composition comprising a pharmaceutically acceptable carrier and at least one ingredient selected from the group consisting of (a) to (e) below: (a) one or more types of peptides as described herein; (b) one or more types of polynucleotides encoding the peptide(s) as described herein in an expressible form; (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide as described herein and an HLA antigen; (d) an exosome that presents on its cell surface a complex of the peptide as described herein and an HLA antigen; and (e) a CTL that targets the peptide as described herein. In another aspect, the present disclosure refers to a method of inducing an APC(s) having CTL-inducing ability, which comprises a step selected from the group consisting of: contacting an APC(s) with the peptide as described herein *in vitro*, *ex vivo* or *in vivo*; and introducing a polynucleotide encoding the peptide as described herein into an APC(s). In another aspect, the present disclosure refers to a method of inducing a CTL(s), which comprises a step selected from the group consisting of: (a) co-culturing a CD8-positive T cell(s) with an APC(s) that presents on its surface a complex of an HLA antigen and the peptide as described herein; (b) co-culturing a CD8-positive T cell(s) with an exosome(s) that presents on its surface a complex of an HLA antigen and the peptide as described herein; and (c) introducing into a CD8-positive T cell(s) a polynucleotide encoding each subunit of a T cell receptor (TCR) capable of binding to the peptide as described herein presented by an HLA antigen on a cell surface. In yet another aspect, the present disclosure refers to an APC that presents on its surface

a complex of an HLA antigen and the peptide as described herein. In yet another aspect, the present disclosure refers to a CTL that targets the peptide as described herein. In yet another aspect, the present disclosure refers to a method of inducing an immune response against cancer, which comprises administering to a subject at least one ingredient selected from the group consisting of (a) to (e) below: (a) one or more types of peptides as described herein; (b) one or more types of polynucleotides encoding the peptide(s) as described herein in an expressible form; (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide as described herein 3 and an HLA antigen; (d) an exosome that presents on its cell surface a complex of the peptide as described herein and an HLA antigen; and (e) a CTL that targets the peptide as described herein. In yet another aspect, the present disclosure refers to a method of treating and/or preventing cancer, and/or preventing postoperative recurrence thereof, which comprises administering to a subject at least one ingredient selected from the group consisting of (a) to (e) below: (a) one or more types of peptides as described herein; (b) one or more types of polynucleotides encoding the peptide(s) as described herein in an expressible form; (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide as described herein and an HLA antigen; d) an exosome that presents on its cell surface a complex of the peptide as described herein and an HLA antigen; and (e) a CTL that targets the peptide as described herein. In yet another aspect, the present disclosure refers to an antibody that binds to the peptide as described herein. In yet another aspect, the present disclosure refers to a method of screening for a peptide having CTL-inducing ability, which comprises the steps of: (a) generating candidate sequences consisting of an amino acid sequence in which one, two or several amino acid residues are substituted, deleted, inserted and/or added to an original amino acid sequence consisting of the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37; (b) selecting from among the candidate sequences generated in (a), a candidate sequence that does not have significant homology (sequence identity) with any known human gene product other than CDCA1; (c) contacting an APC with a peptide consisting of the candidate sequence selected in (b); (d) contacting the APC of (c) with a CD8-positive T cell; and (e) selecting a peptide having an equal to or higher CTL-inducing ability than that of a peptide consisting of the original amino acid sequence. In yet another aspect, the present disclosure refers to an emulsion comprising one or more types of peptides as described herein, a water-soluble carrier and an oil adjuvant. In yet another aspect, the present disclosure refers to a kit comprising a container that houses the composition as described herein and a container that houses an adjuvant.

The peptides of the present disclosure encompass peptides in which one, two, or more amino acid(s) is/are substituted, deleted, inserted and/or added, as long as the resultant modified peptides retain the CTL-inducing ability of the original peptide.

The present disclosure further provides isolated polynucleotides encoding any one of the peptides of the present disclosure. Similar to the peptides of the present disclosure, these polynucleotides can be used for inducing APCs with CTL-inducing ability, and can be administered to subjects for inducing an immune response against CDCA1-expressing cancer cells.

[0011]

The present disclosure also provides compositions comprising one or more types of peptides of the present disclosure, one or more types of polynucleotides encoding one or more types of peptides of the present disclosure, APCs of the present disclosure, exosomes presenting peptides of the present disclosure, and/or CTLs of the present disclosure. The compositions of the present disclosure are preferably pharmaceutical compositions. The pharmaceutical compositions of the present disclosure can be used for treating and/or preventing cancer, as well as preventing postoperative recurrence thereof. They can also be used for inducing an immune response against cancer. When administered to a subject, a peptide of the present disclosure is presented on the surface of an APC, and as a result CTLs targeting the peptide are induced. Therefore, another objective of the present disclosure is to provide compositions for inducing CTLs, wherein the compositions comprise one or more types of peptides of the present disclosure, one or more types of polynucleotides encoding one or more types of peptides of the present disclosure, APCs of the present disclosure, and/or exosomes presenting peptides of the present disclosure.

[0012]

A further objective of the present disclosure is to provide methods of inducing APCs having CTL-inducing ability, wherein the methods comprise a step of contacting one or more types of peptides of the present disclosure with an APC, or a step of introducing a polynucleotide encoding any one peptide of the present disclosure into an APC.

[0013]

The present disclosure further provides a method of inducing CTLs, comprising a step of co-culturing a CD8-positive T cell with an APC that presents on its surface a complex of an HLA antigen and a peptide of the present disclosure, a step of co-culturing a CD8-positive T cell with an exosome that presents on its surface a complex of an HLA antigen and a peptide of the present disclosure, or a step of introducing into a CD8-positive T cell a vector comprising a polynucleotide encoding each subunit of a T cell receptor (TCR) capable of binding to a peptide of the present disclosure presented by an HLA antigen on a cell surface.

[0014]

A further objective of the present disclosure is to provide isolated APCs that present on their surface a complex of an HLA antigen and a peptide of the present disclosure. The present disclosure further provides isolated CTLs targeting a peptide of the present disclosure. These APCs and CTLs can be used in immunotherapy for CDCA1-expressing cancers.

[0015]

Another objective of the present disclosure is to provide methods of inducing an immune response against cancer in a subject, wherein the methods comprise a step of administering to the subject a composition(s) comprising a peptide(s) of the present disclosure or a polynucleotide(s) encoding the peptide(s), an APC(s) of the present disclosure , an exosome(s) presenting a peptide(s) of the present disclosure, and/or a CTL(s) of the present disclosure. Another objective of the present disclosure is to provide methods of treating and/or preventing cancer, as well as preventing postoperative recurrence thereof in a subject, wherein the methods comprise a step of administering to the subject a peptide(s) of the present disclosure, a polynucleotide(s) encoding the peptide(s), an APC(s) of the present disclosure, an exosome(s) presenting a peptide(s) of the present disclosure, and/or a CTL(s) of the present disclosure.

[0016]

In addition to the above, other objects and features of the present disclosure will become more fully apparent when the following detailed description is read in conjunction with the accompanying figures and examples. However, it is to be understood that both the foregoing summary of the present disclosure and the following detailed description are of exemplified examples, and not restrictive of the present disclosure or other alternate examples of the present disclosure. In particular, while the present disclosure is described herein with reference to a number of specific examples, it will be appreciated that the description is illustrative of the present disclosure and is not constructed as limiting of the present disclosure. Various modifications and applications may occur to those who are skilled in the art, without departing from the spirit and the scope of the present disclosure, as described by the appended claims. Likewise, other objects, features, benefits and advantages of the present disclosure will be apparent from this summary and certain examples described below, and will be readily apparent to those skilled in the art. Such objects, features, benefits and advantages will be apparent from the above in conjunction with the accompanying examples, data, figures and all reasonable inferences to be drawn therefrom, alone or with consideration of the references incorporated herein.

Brief Description of the Drawings

[0017]

Fig. 1 is comprised of photos (a) to (j) showing the results of the IFN-gamma enzyme-linked immunospot (ELISPOT) assay performed using cells induced using peptides derived from CDCA1. In the figure, "+" shows IFN-gamma production against target cells pulsed with a peptide of interest and "-" shows IFN-gamma production against target cells that have not been pulsed with any peptides (negative control). In comparison with the negative control, peptide-specific IFN-gamma production was observed in:
Well #1 with CDCA1-A01-8-138 (SEQ ID NO: 1) (a),
Well #7 with CDCA1-A01-9-290 (SEQ ID NO: 25) (b),
Well #5 with CDCA1-A01-9-130 (SEQ ID NO: 33) (c),
Well #7 with CDCA1-A01-9-246 (SEQ ID NO: 34) (d),
Well #1 with CDCA1-A01-9-268 (SEQ ID NO: 35) (e),
Well #3 with CDCA1-A01-9-288 (SEQ ID NO: 37) (f),
Well #2 with CDCA1-A01-10-136 (SEQ ID NO: 8) (g),
Well #6 with CDCA1-A01-10-56 (SEQ ID NO: 10) (h), and
Well #6 with CDCA1-A01-10-48 (SEQ ID NO: 13) (i).
CTL clones were established from the boxed cells in the photos that showed a reaction. In contrast, CDCA1-A01-10-66 (SEQ ID NO: 9) (j) is shown as an example of typical negative data where peptide-specific IFN-gamma production was not observed.

[0018]

Fig. 2 is comprised of a series of line graphs (a) to (c) showing IFN-gamma production of CTL clones established by limiting dilution method following induction by CDCA1-A01-10-136 (SEQ ID NO: 8), CDCA1-A01-10-56 (SEQ ID NO: 10), or CDCA1-A01-10-48 (SEQ ID NO: 13). These results show peptide-specific IFN-gamma production of the CTL clones. In the figure, "+" shows IFN-gamma production of the CTL clones against target cells pulsed with a peptide of interest and "-" shows IFN-gamma production of the CTL clones against target cells that have not been pulsed with any peptides. The R/S ratio indicates the ratio of the cell number

Nat Med. 2002; 8(6): 631-7, and such). The complexes of the present invention, for example, can be used in the quantification of CTLs specific to a peptide of the present invention. For example, a blood sample is collected from a subject administered with a pharmaceutical composition of the present invention, and CD4-negative cells are prepared after separation of PBMCs and contacted with a fluorescent dye-conjugated complex of the present invention. Then, the percentage of CTLs specific to a peptide of the present invention can be measured by flow cytometry analysis. For example, immune response-inducing effects by a pharmaceutical composition of the present invention can be monitored by measuring the specific CTLs against a peptide of the present invention before, during and/or after administration of the pharmaceutical composition of the present invention.

[0169]

XII. Antibodies

The present invention further provides antibodies that bind to the peptide of the present invention. Preferable antibodies bind specifically to a peptide of the present invention, but do not bind (or weakly bind) to one that is not the peptide of the present invention. The binding specificity of an antibody can be confirmed by inhibition assay. That is, if the binding between an antibody to be analyzed and a full-length CDCA1 polypeptide is inhibited in the presence of a peptide of the present invention, this antibody is shown to specifically bind to the peptide of the present invention. Antibodies against peptides of the present invention can be used in assays of disease diagnosis and prognosis, as well as subject selection for administration of the pharmaceutical compositions of the present invention and monitoring of the pharmaceutical compositions of the present invention.

[0170]

The present invention also provides various immunological assays for detecting and/or quantifying peptides of the present invention or fragments thereof. Such immunological assays include radioimmunoassay, immunochromatography, enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunofluorescence assay (ELIFA) and such, without being limited thereto, and are performed within the scope of the various immunological assay formats well known in the art.

[0171]

The antibodies of the present invention can be used in immunological imaging methods that can detect CDCA1-epxressing diseases, and examples thereof include radioactive scintigraphic imaging using a labelled antibody of the present invention, without being limited thereto. Such assay methods are used clinically in the detection, monitoring, and prognosis of CDCA1-expressing cancers; and examples of such cancer include bladder cancer, breast cancer, cervical cancer, cholangiocellular cancer, chronic myeloid leukemia (CML), esophagus cancer,

Claims

1. A peptide of less than 15 amino acids having cytotoxic T cell (CTL)-inducing ability, which comprises the amino acid sequence selected from the group below:

(a) the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37; and

(b) the amino acid sequence in which one, two, three or several amino acids are substituted, deleted, inserted and/or added to the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37.

2. The peptide of claim 1, comprising the amino acid sequence in which one or more substitution(s) selected from (a) to (c) below is introduced into the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37:

(a) the second amino acid from the N terminus is substituted with an amino acid selected from the group consisting of threonine and serine;

(b) the third amino acid from the N terminus is substituted with an amino acid selected from the group consisting of aspartic acid and glutamic acid; and

(c) the C-terminal amino acid is substituted with tyrosine.

3. The peptide of claim 1, which consists of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37.

4. A polynucleotide, which encodes the peptide of any one of claims 1 to 3.

5. A composition comprising a pharmaceutically acceptable carrier and at least one ingredient selected from the group consisting of (a) to (e) below:

(a) one or more types of peptides of any one of claims 1 to 3;

(b) one or more types of polynucleotides encoding the peptide(s) of any one of claims 1 to 3 in an expressible form;

(c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of claims 1 to 3 and an HLA antigen;

(d) an exosome that presents on its cell surface a complex of the peptide of any one of claims 1 to 3 and an HLA antigen; and

(e) a CTL that targets the peptide of any one of claims 1 to 3.

6. The composition of claim 5, which is a composition for inducing a CTL(s), wherein the ingredient is at least one ingredient selected from the group consisting of (a) to (d) below:

(a) one or more types of peptides of any one of claims 1 to 3;

(b) one or more types of polynucleotides encoding the peptide(s) of any one of claims 1 to 3 in an expressible form;

(c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of claims 1 to 3 and an HLA antigen; and

(d) an exosome that presents on its cell surface a complex of the peptide of any one of claims 1 to 3 and an HLA antigen.

7. The composition of claim 5, which is a pharmaceutical composition.

8. The composition of claim 7, which is for one or more uses selected from the group consisting of (i) cancer treatment, (ii) cancer prevention (prophylaxis) and (iii) prevention (prophylaxis) of postoperative cancer recurrence.

9. The composition of claim 7, which is for inducing an immune response against cancer.

10. The composition of claim 8 or 9, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangiocellular cancer, chronic myeloid leukemia (CML), esophagus cancer, gastric cancer, non-small-cell lung cancer, lymphoma, osteosarcoma, prostate cancer, kidney cancer, small-cell lung cancer, head and neck cancer, soft tissue tumor and colon cancer.

11. The composition of any one of claims 5 to 10, which is formulated for administration to a subject positive for HLA-A01.

12. A method of inducing an APC(s) having CTL-inducing ability, which comprises a step selected from the group consisting of:

(a) contacting an APC(s) with the peptide of any one of claims 1 to 3 *in vitro*, *ex vivo* or *in vivo*; and

(b) introducing a polynucleotide encoding the peptide of any one of claims 1 to 3 into an APC(s).

13. A method of inducing a CTL(s), which comprises a step selected from the group consisting of:

(a) co-culturing a CD8-positive T cell(s) with an APC(s) that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 3;
(b) co-culturing a CD8-positive T cell(s) with an exosome(s) that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 3; and
(c) introducing into a CD8-positive T cell(s) a polynucleotide encoding each subunit of a T cell receptor (TCR) capable of binding to the peptide of any one of claims 1 to 3 presented by an HLA antigen on a cell surface.

14. An APC that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 3.

15. The APC of claim 14, which is induced by the method of claim 12.

16. A CTL that targets the peptide of any one of claims 1 to 3.

17. The CTL of claim 16, which is induced by the method of claim 13.

18. A method of inducing an immune response against cancer, which comprises administering to a subject at least one ingredient selected from the group consisting of (a) to (e) below:
(a) one or more types of peptides of any one of claims 1 to 3;
(b) one or more types of polynucleotides encoding the peptide(s) of any one of claims 1 to 3 in an expressible form;
(c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of claims 1 to 3 and an HLA antigen;
(d) an exosome that presents on its cell surface a complex of the peptide of any one of claims 1 to 3 and an HLA antigen; and
(e) a CTL that targets the peptide of any one of claims 1 to 3.

19. A method of treating and/or preventing cancer, and/or preventing postoperative recurrence thereof, which comprises administering to a subject at least one ingredient selected from the group consisting of (a) to (e) below:
(a) one or more types of peptides of any one of claims 1 to 3;
(b) one or more types of polynucleotides encoding the peptide(s) of any one of claims 1 to 3 in an expressible form;
(c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of claims 1 to 3 and an HLA antigen;

(d) an exosome that presents on its cell surface a complex of the peptide of any one of claims 1 to 3 and an HLA antigen; and

(e) a CTL that targets the peptide of any one of claims 1 to 3.

20. An antibody that binds to the peptide of any one of claims 1 to 3.

21. A method of screening for a peptide having CTL-inducing ability, which comprises the steps of:

(a) generating candidate sequences consisting of an amino acid sequence in which one, two or several amino acid residues are substituted, deleted, inserted and/or added to an original amino acid sequence consisting of the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37;

(b) selecting from among the candidate sequences generated in (a), a candidate sequence that does not have significant homology (sequence identity) with any known human gene product other than CDCA1;

(c) contacting an APC with a peptide consisting of the candidate sequence selected in (b);

(d) contacting the APC of (c) with a CD8-positive T cell; and

(e) selecting a peptide having an equal to or higher CTL-inducing ability than that of a peptide consisting of the original amino acid sequence.

22. An emulsion comprising one or more types of peptides of any one of claims 1 to 3, a water-soluble carrier and an oil adjuvant.

23. A kit comprising a container that houses the composition of any one of claims 5 to 11 and a container that houses an adjuvant.