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Summary

[0011]

In one aspect, the present invention refers to an isolated peptide of less than 15 amino acids having an ability to induce a cytotoxic T lymphocyte(s) (CTL(s)), wherein the peptide comprises an amino acid sequence selected from (a) or (b) below:
(a) an amino acid sequence of SEQ ID NO: 7; (b) an amino acid sequence in which 1 or 2 amino acid(s) are substituted and/or added in the amino acid sequence of SEQ ID NO: 7. In another aspect, the present invention refers to an isolated polynucleotide encoding the peptide the present invention. In another aspect, the present invention refers to a composition comprising at least one active ingredient selected from the group consisting of: (a) the peptide of the present invention; (b) the polynucleotide of the present invention; (c) an antigen-presenting cell (APC) that presents the peptide of the present invention on its surface; (d) an exosome that presents the peptide of the present invention on its surface; and (e) a CTL that targets the peptide of the present invention. In yet another aspect, the present invention refers to a pharmaceutical composition for use in the treatment and/or prophylaxis of cancer, and/or the prevention of a postoperative recurrence thereof, wherein the composition comprises at least one active ingredient selected from the group consisting of: (a) the peptide of the present invention; (b) the polynucleotide of the present invention; (c) an APC that presents the peptide of the present invention on its surface; (d) an exosome that presents the peptide of the present invention on its surface; and (e) a CTL that can recognize a cell presenting the peptide of the present invention. In yet another aspect, the present invention refers to an *in vitro* method for inducing an APC having an ability to induce CTL(s), wherein the method comprises the step selected from the group consisting of: (a) contacting an APC with the peptide of the present invention, and (b) introducing a polynucleotide encoding the peptide of the present invention into an APC. In yet another aspect, the present invention refers to An *in vitro* method for inducing a CTL, wherein the method comprises a step selected from the group consisting of: (a) co-culturing a CD8 positive T cell with an APC that presents on its surface a complex of an HLA antigen and the peptide of the present invention; (b) co-culturing a CD8 positive T cell with an exosome that presents on its surface a complex of an HLA antigen and the peptide of the present invention; and (c) introducing into a CD8 positive T cell a polynucleotide encoding both of T cell receptor (TCR) subunits or polynucleotides encoding each of TCR subunits, wherein the TCR formed by said subunits can bind to a complex of the peptide of the present invention and an HLA antigen on a cell surface. In yet another aspect, the present invention refers to an isolated APC that presents on its surface a complex of an HLA antigen and the peptide of the present invention. In yet another aspect, the present invention refers to an isolated CTL that targets the peptide of the present invention, which is preferably induced by the method of the present

invention. In yet another aspect, the present invention refers to an antibody or immunologically active fragment thereof which specifically binds to the peptide of the present invention. In yet another aspect, the present invention refers to a method of screening for a peptide having an ability to induce a CTL that has specific cytotoxic activity against a cell that presents a fragment derived from KNTC2, wherein the method comprises the steps of: (i) providing a candidate sequence consisting of an amino acid sequence modified by substituting, deleting, inserting and/or adding one, two or several amino acid residues to an original amino acid sequence, wherein the original amino acid sequence is SEQ ID NO: 7; (ii) selecting a candidate sequence that does not have homology with the peptides derived from any known human gene products other than KNTC2; (iii) contacting a peptide consisting of the candidate sequence selected in step (ii) with an antigen presenting cell; (iv) contacting the antigen presenting cell of step (iii) with a CD8 positive T cell; and (v) identifying the peptide of which ability to induce CTL(s) is same to or higher than a peptide consisting of the original amino acid sequence.

The present invention is based, at least in part, on the discovery of novel peptides that may serve as suitable targets of immunotherapy. Because TAAs are generally perceived by the immune system as "self" and therefore often have no innate immunogenicity, the discovery of appropriate targets is still of importance. In the course of the present invention, KNTC2 (a typical amino acid sequence shown in SEQ ID NO: 79; a typical nucleotide sequence shown in SEQ ID NO: 77 (GenBank Accession No. NM_006101) or SEQ ID NO 78 (GenBank Accession No. AF017790)) is demonstrated to be specifically over-expressed in cancers, examples of which include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, chronic myeloid leukemia (CML), colorectal cancer, esophageal cancer, non-small cell lung cancer (NSCLC), lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor. Thus, the present invention focuses on KNTC2 as a candidate target for cancer/tumor immunotherapy.

[0012]

To that end, the present invention is directed, at least in part, to the identification of specific epitope peptides that possess the ability to induce cytotoxic T lymphocytes (CTLs) specific to KNTC2 among peptides derived from KNTC2.

[0013]

The results disclosed herein demonstrate that identified peptides are HLA-A2 restricted epitope peptides that can induce potent and specific immune responses against cells expressing KNTC2.

[0014]

Accordingly, it is an object of the present invention to provide KNTC2-derived peptides that can be used to induce CTLs in vitro, ex vivo or in vivo in HLA-A2 restricted manner, or to be directly administered to a subject so as to induce in vivo immune responses against cancers, examples of which include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, chronic myeloid leukemia (CML), colorectal cancer, esophageal cancer, non-small cell lung cancer (NSCLC), lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor.

[0015]
The peptides of the present disclosure are generally less than 15, 14, 13, 12, 11, or 10 amino acids in length. Preferred peptides are nonapeptides and decapeptides. Particularly preferred nonapeptides and decapeptides have an amino acid sequence selected from among SEQ ID NOs: 2, 3, 7, 17, 41, 53 and 68.
[0016]
The present disclosure also contemplates modified peptides having an amino acid sequence in which one, two or more amino acids are substituted, deleted, inserted and/or added to an amino acid sequence selected from among SEQ ID NOs: 2, 3, 7, 17, 41, 53 and 68, provided the resulting modified peptides retain the requisite CTL inducibility of the original unmodified peptide.
[0017]
In one example, when the original peptide is a 9-mer (e.g., one of SEQ ID NOs: 2, 3, 7, and 17), the size of the modified peptide is preferably in the range of 9 to 40 amino acids, such as in the range of 9 to 20 amino acids, for example in the range of 9 to 15 amino acids. Likewise, when the original peptide is a 10-mer (e.g., one of SEQ ID NOs: 41, 53 and 68), the size of the modified peptide is preferably in the range of 10 to 40 amino acids, such as in the range of 10 to 20 amino acids, for example in the range of 10 to 15 amino acids.
[0018]
The present disclosure further encompasses isolated polynucleotides encoding any one of the peptides of the present disclosure. These polynucleotides can be used to induce or prepare antigen presenting cells (APCs) having CTL inducibility. Like the peptides of the present disclosure, such APCs can be administered to a subject so as to induce an immune response against a cancer.
[0019]
When administered to a subject, the peptides of the present disclosure can be presented on the surface of APCs so as to induce CTLs targeting the respective peptides. Therefore, one object of the present invention is to provide agents or compositions including one or more peptides of the present disclosure, or one or more polynucleotides encoding such peptides. The composition of the present disclosure may be used to induce a CTL and thus find utility in the treatment and/or prophylaxis of a cancer, and/or the prevention of a metastasis or post-operative recurrence thereof. Examples of targeted cancers contemplated by the present invention include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, chronic myeloid leukemia (CML), colorectal cancer, esophageal cancer, non-small cell lung cancer (NSCLC), lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor.
[0020]
The present disclosure further contemplates pharmaceutical compositions that include one or more peptides or polynucleotides of the present disclosure. The pharmaceutical composition is preferably formulated for use in the treatment and/or prophylaxis of a cancer, more particularly a primary cancer, and/or prevention of metastatic or post-

operative recurrence thereof. Instead of or in addition to the peptides or polynucleotides of the present disclosure, the pharmaceutical compositions of the present disclosure may include as active ingredients APCs or exosomes that present any of the peptides of the present disclosure.

[0021]

The peptides or polynucleotides of the present disclosure may be used to induce APCs that present on the surface a complex of a human leukocyte antigen (HLA) and a peptide of the present disclosure, for example, by contacting APCs derived from a subject with a peptide of the present disclosure or by introducing a polynucleotide encoding a peptide of the present disclosure into APCs. Such APCs have the ability to induce CTLs that specifically recognize cells that present target peptides on the surface and thus are useful in the context of cancer immunotherapy. Accordingly, the present disclosure encompasses the methods for inducing APCs with CTL inducibility as well as the APCs obtained by such methods.

[0022]

In addition, the present disclosure also encompasses compositions that induce APCs having CTL inducibility, such compositions including any peptides or polynucleotides of the present disclosure.

[0023]

It is a further object of the present disclosure to provide methods for inducing CTLs, such methods including the step of co-culturing CD8 positive T cells with APCs presenting on its surface a complex of an HLA antigen and a peptide of the present disclosure, the step of co-culturing CD8 positive T cells with exosomes presenting on its surface a complex of an HLA antigen and a peptide of the present disclosure, or the step of introducing a polynucleotide encoding both of T cell receptor (TCR) subunits or polynucleotides encoding each of TCR subunits, wherein the TCR can bind to a complex of the peptide of the present disclosure and an HLA antigen presented on cell surface. CTLs obtained by such methods can find use in the treatment and/or prevention of cancers, examples of which include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, chronic myeloid leukemia (CML), colorectal cancer, esophageal cancer, non-small cell lung cancer (NSCLC), lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor. Accordingly, the present disclosure encompasses both the methods for inducing CTLs and the CTLs obtained by such methods.

[0024]

Yet another object of the present disclosure is to provide isolated APCs that present on the surface a complex of an HLA antigen and a peptide of the present disclosure. The present disclosure further provides isolated CTLs that target peptides of the present disclosure. Such CTLs may be also defined as CTLs that can recognize (or bind to) a complex of a peptide of the present disclosure and an HLA antigen on the cell surface. These APCs and CTLs find utlity in the context of cancer immunotherapy.

[0025]
It is yet another object of the present disclosure to provide methods for inducing an immune response against a cancer in a subject in need thereof, such methods including the step of administering to the subject a composition that includes at least one component selected from among (a) a peptide of the present disclosure or a polynucleotide encoding such a peptide, (b) an APC or exosome presenting such peptide(s) and (c) a CTL that can recognize a cell presenting a peptide of the present disclosure on its surface.
[0026]
One example of the present disclosure pertains to a peptide of the present disclosure, an agent or composition containing such a peptide for use as a medicament.
[0027]
The applicability of the present disclosure extends to any of a number of diseases relating to or arising from KNTC2 overexpression, such as cancer, examples of which include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, chronic myeloid leukemia (CML), colorectal cancer, esophageal cancer, non-small cell lung cancer (NSCLC), lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor.
[0028]
More specifically, the present disclosure provides followings:
[1] An isolated peptide having cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide comprises an amino acid sequence (a) or (b) bellow:
(a) an amino acid sequence of an immunologically active fragment of KNTC2;
(b) an amino acid sequence in which 1, 2, or several amino acid(s) are substituted, deleted, inserted and/or added in an amino acid sequence of an immunologically active fragment of KNTC2,
wherein the CTL induced by the peptide has specific cytotoxic activity against a cell that presents a fragment derived from KNTC2;
[2] The peptide of [1], wherein the peptide comprises an amino acid sequence (a) or (b) bellow:
(a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 7, 17, 41, 53 and 68;
(b) an amino acid sequence in which 1, 2, or several amino acid(s) are substituted, deleted, inserted and/or added in the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 7, 17, 41, 53 and 68;
[3] The peptide of [2], wherein the peptide has one or both of the following characteristics: (a) the second amino acid from the N-terminus is leucine or methionine; and
(b) the C-terminal amino acid is valine or leucine;
[4] The peptide of any one of [1] to [3], wherein the peptide is a nonapeptide or a decapeptide;

[14] An isolated CTL that targets the peptide of any one of [1] to [5];
[15] The CTL of [14], which is induced by the method of [11];
[16] A method of inducing an immune response against cancer in a subject, wherein the method comprises the step of administering to the subject a composition comprising the peptide of any one of [1] to [5], or a polynucleotide encoding the peptide;
[17] An antibody or immunologically active fragment thereof against the peptide of any one of [1] to [5];
[18] A vector comprising a nucleotide sequence encoding the peptide of any one of [1] to [5];
[19] A host cell transformed or transfected with a vector of [18];
[20] A diagnostic kit comprising the peptide of any one of [1] to [5, the polynucleotide of [6] or the antibody or immunologically active fragment of [17]; and
[21] A method of screening for a peptide having an ability to induce a CTL that has specific cytotoxic activity against a cell that presents a fragment derived from KNTC2, wherein the method comprises the steps of:
(i) providing a candidate sequence consisting of an amino acid sequence modified by substituting, deleting, inserting and/or adding one, two or several amino acid residues to an original amino acid sequence, wherein the original amino acid sequence is selected from the group consisting of SEQ ID NOs: 2, 3, 7, 17, 41, 53 and 68;
(ii) selecting a candidate sequence that does not have substantial significant homology (or sequence identity) with the peptides derived from any known human gene products other than KNTC2;
(iii) contacting a peptide consisting of the candidate sequence selected in step (ii) with an antigen presenting cell;
(iv) contacting the antigen presenting cell of step (iii) with a CD8 positive T cell; and
(v) identifying the peptide of which CTL inducibility is same to or higher than a peptide consisting of the original amino acid sequence.
[22] A pharmaceutical composition comprising a peptide of any one of [1] to [5];
[23] A peptide of any one of [1] to [5] for use as a medicament; and
[24] A polynucleotide of [6] or a vector of [18] for use as a medicament.
[0029]
Alternatively, the present application also provides following examples:
[1] An isolated peptide having cytotoxic T lymphocyte (CTL) inducibility, wherein the CTL induced by the peptide have specific cytotoxic activity against a cell that presents a fragment derived from KNTC2, further wherein the peptide has an amino acid sequence (a) or (b) below:
(a) an amino acid sequence of an immunologically active fragment of KNTC2;
(b) an amino acid sequence in which 1, 2, or several amino acid(s) are substituted,

CLAIMS

1. An isolated peptide of less than 15 amino acids having an ability to induce a cytotoxic T lymphocyte(s) (CTL(s)), wherein the peptide comprises an amino acid sequence selected from (a) or (b) below:
   (a) an amino acid sequence of SEQ ID NO: 7;
   (b) an amino acid sequence in which 1 or 2 amino acid(s) are substituted and/or added in the amino acid sequence of SEQ ID NO: 7.

2. The peptide of claim 1, wherein the peptide has one or both of the following characteristics:
   (a) the second amino acid from the N-terminus of the amino acid sequence of SEQ ID NO: 7 is substituted with methionine; and
   (b) the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 7 is substituted with valine or leucine.

3. The peptide of claim 1, wherein the peptide consists of the amino acid sequence of SEQ ID NO: 7.

4. An isolated polynucleotide encoding the peptide of any one of claims 1 to 3.

5. A composition comprising at least one active ingredient selected from the group consisting of:
   (a) the peptide of any one of claims 1 to 3;
   (b) the polynucleotide of claim 4;
   (c) an antigen-presenting cell (APC) that presents the peptide of any one of claims 1 to 3 on its surface;
   (d) an exosome that presents the peptide of any one of claims 1 to 3 on its surface; and
   (e) a CTL that targets the peptide of any one of claims 1 to 3.

6. A pharmaceutical composition for use in the treatment and/or prophylaxis of cancer, and/or the prevention of a postoperative recurrence thereof, wherein the composition comprises at least one active ingredient selected from the group consisting of:
   (a) the peptide of any one of claims 1 to 3;
   (b) the polynucleotide of claim 4;
   (c) an APC that presents the peptide of any one of claims 1 to 3 on its surface;
   (d) an exosome that presents the peptide of any one of claims 1 to 3 on its surface; and
   (e) a CTL that can recognize a cell presenting the peptide of any one of claims 1 to 3.

7. The pharmaceutical composition for use according to claim 6, wherein the pharmaceutical composition is formulated for the administration to a subject whose HLA antigen is HLA-A2.

8. An *in vitro* method for inducing an APC having an ability to induce CTL(s), wherein the method comprises the step selected from the group consisting of:
   (a) contacting an APC with the peptide of any one of claims 1 to 3, and
   (b) introducing a polynucleotide encoding the peptide of any one of claims 1 to 3 into an APC.

9. An *in vitro* method for inducing a CTL, wherein the method comprises a step selected from the group consisting of:
   (a) co-culturing a CD8 positive T cell with an APC 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 with an exosome 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 a polynucleotide encoding both of T cell receptor (TCR) subunits or polynucleotides encoding each of TCR subunits, wherein the TCR formed by said subunits can bind to a complex of the peptide of any one of claims 1 to 3 and an HLA antigen on a cell surface.

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

11. The APC of claim 10, which is induced by the method of claim 8.

12. An isolated CTL that targets the peptide of any one of claims 1 to 3, which is preferably induced by the method of claim 9.

13. The peptide of any one of claims 1 to 3, or a polynucleotide encoding the peptide for use in the treatment and/or prevention of cancers or tumors, and/or prevention of a post-operative recurrence thereof.

14. An antibody or immunologically active fragment thereof which specifically binds to the peptide of any one of claims 1 to 3.

15. A method of screening for a peptide having an ability to induce a CTL that has specific cytotoxic activity against a cell that presents a fragment derived from KNTC2, wherein the method comprises the steps of:
    (i) providing a candidate sequence consisting of an amino acid sequence modified by substituting, deleting, inserting and/or adding one, two or several amino acid residues to an original amino acid sequence, wherein the original amino acid sequence is SEQ ID NO: 7;
    (ii) selecting a candidate sequence that does not have homology with the peptides derived from any known human gene products other than KNTC2;
    (iii) contacting a peptide consisting of the candidate sequence selected in step (ii) with an antigen presenting cell;
    (iv) contacting the antigen presenting cell of step (iii) with a CD8 positive T cell; and
    (v) identifying the peptide of which ability to induce CTL(s) is same to or higher than a peptide consisting of the original amino acid sequence.