vaccine trial have shown only a low objective response rate (NPL 11, Belli F et al., J Clin Oncol 2002 Oct 15, 20(20): 4169-80; NPL 12, Coulie PG et al., Immunol Rev 2002 Oct, 188: 33-42; NPL 13, Rosenberg SA et al., Nat Med 2004 Sep, 10(9): 909-15). Accordingly, there remains a need for new TAAs as immunotherapeutic targets.

[0005] Epithelial cell transforming sequence 2 (ECT2) oncogene (GenBank Accession No. AY376439; e.g., SEQ ID No: 41) has been discovered from the transcripts identified as up-regulated in cancer. The ECT2 gene is specifically up-regulated in the tumor cells of various cancers, including, but 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, pancreatic cancer, prostate cancer, renal carcinoma and small cell lung cancer (SCLC) and thus is of particular to the present invention (PTL 1, WO2007/013671; PTL 2, WO2008/102557; PTL 3, WO2010/007791). In particular, immunogenic peptides derived from ECT2 may find utility in selectively killing tumor cells that express such antigens.

Citation List

Non Patent Literature

[0006] [NPL 1] Boon T, Int J Cancer 1993 May 8, 54(2): 177-80
[NPL 2] Boon T & van der Bruggen P, J Exp Med 1996 Mar 1, 183(3): 725-9
[NPL 3] Harris CC, J Natl Cancer Inst 1996 Oct 16, 88(20): 1442-55
[NPL 4] Butterfield LH et al., Cancer Res 1999 Jul 1, 59(13): 3134-42
[NPL 5] Vissers JL et al., Cancer Res 1999 Nov 1, 59(21): 5554-9
[NPL 6] van der Burg SH et al., J Immunol 1996 May 1, 156(9): 3308-14
[NPL 7] Tanaka F et al., Cancer Res 1997 Oct 15, 57(20): 4465-8
[NPL 8] Fujie T et al., Int J Cancer 1999 Jan 18, 80(2): 169-72
[NPL 9] Kikuchi M et al., Int J Cancer 1999 May 5, 81(3): 459-66
[NPL 10] Oiso M et al., Int J Cancer 1999 May 5, 81(3): 387-94
[NPL 11] Belli F et al., J Clin Oncol 2002 Oct 15, 20(20): 4169-80
[NPL 12] Coulie PG et al., Immunol Rev 2002 Oct, 188: 33-42
[NPL 13] Rosenberg SA et al., Nat Med 2004 Sep, 10(9): 909-15

Patent Literature

[0007] [PTL 1] WO2007/013671
[PTL 2] WO2008/102557
[PTL 3] WO2010/007791

Summary ~~of Invention~~

[0008] The present ~~invention~~disclosure is based, at least in part, on the discovery of the suitable targets

of immunotherapy. Because TAAs are generally perceived by the immune system as "self" and therefore often have no immunogenicity, the discovery of appropriate targets is of extreme importance. Recognizing that ECT2 (SEQ ID NO: 42 encoded by the gene of GenBank Accession No. AY376439 (for example, SEQ ID NO: 41)) has been identified as up-regulated in cancers, examples of which include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophageal cancer, NSCLC, lymphoma, pancreatic cancer, prostate cancer, renal carcinoma and SCLC, the present ~~invention~~disclosure focuses on ECT2 as a candidate for the target of cancer/tumor immunotherapy, more particularly novel ECT2 epitope peptides that may serve as suitable immunotherapeutic targets.

[0009] To that end, the present ~~invention~~disclosure is directed, at least in part, to the identification of specific epitope peptides among the gene products of ECT2 that possess the ability to induce CTLs specific to ECT2. As discussed in detail below, peripheral blood mononuclear cells (PBMCs) obtained from a healthy donor were stimulated using HLA-A*0201 binding candidate peptides derived from ECT2. CTL lines were then established with specific cytotoxicity against the HLA-A2 positive target cells pulsed with each of candidate peptides. The results herein demonstrate that these peptides are HLA-A2 restricted epitope peptides that may induce potent and specific immune responses against cells expressing ECT2. These results further demonstrate that ECT2 is strongly immunogenic and the epitopes thereof are effective targets for caner/tumor immunotherapy.

[0010] Accordingly, it is an object of the present ~~invention~~disclosure to provide isolated peptides binding to HLA antigen, including ECT2 (SEQ ID NO: 42) and the immunologically active fragments thereof. Such peptides are expected to have CTL inducibility and, thus, can be used to induce CTL ex vivo or to be administered to a subject for inducing immune responses against cancers, examples of which include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophageal cancer, NSCLC, lymphoma, pancreatic cancer, prostate cancer, renal carcinoma and SCLC. Preferred peptides are nonapeptides and decapeptides, more preferably, nonapeptides and decapeptides having the amino acid sequence selected from among SEQ ID NOs: 1 to 40. Peptides having an amino sequence selected among SEQ ID NOs: 1, 3 and 21 showed strong CTL inducibility and thus are particularly preferred.

[0011] The present ~~invention~~disclosure also contemplates modified peptides having an amino acid sequence selected from among SEQ ID NOs: 1 to 40 in which one, two or more amino acids are substituted, inserted, deleted or added, so long as the modified peptides retain the requisite CTL inducibility of the original peptide.

In an aspect, there is provided an isolated peptide of less than 15 amino acids having cytotoxic T lymphocyte (CTL) inducibility selected from the group consisting of:

(a) an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3 and 21, and

(b) an isolated peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3 and 21, in which 1 or 2 amino acid(s) are substituted or added.

The present ~~invention~~disclosure further encompasses isolated polynucleotides that encode any

of the peptides of the present ~~invention~~disclosure. These polynucleotides can be used to induce or prepare APCs with CTL inducibility or, like the above-described peptides of the present ~~invention~~disclosure, can be administered to a subject for inducing immune responses against cancers.

[0012] When administered to a subject, the present peptides are presented on the surface of APCs so as to induce CTLs targeting the respective peptides. Therefore, one object of the present ~~invention~~disclosure is to provide agents, compositions and/or substances that include or incorporate any peptides or polynucleotides of the present ~~invention~~disclosure for inducing CTLs. In an aspect, there is provided a composition for inducing CTL, wherein the composition comprises one or more peptide(s) as defined above. In another aspect, there is provided a pharmaceutical composition for the treatment of cancer over expressing ECT2, wherein the composition comprises one or more peptide(s) as defined above. In another aspect, there is provided a kit comprising any of the peptides as defined above. Such agents, compositions and substances can be used for the treatment and/or prophylaxis and/or post-operative recurrence of cancers, especially bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophageal cancer, NSCLC, lymphoma, pancreatic cancer, prostate cancer, renal carcinoma and SCLC. Thus, it is yet another object of the present ~~invention~~disclosure to provide pharmaceutical agents, compositions and/or substances for the treatment and/or prophylaxis and/or prevention of postoperative recurrence of cancer that include or incorporate one or more peptides or polynucleotides of the present ~~invention~~disclosure. Instead of or in addition to the present peptides or polynucleotides, the pharmaceutical agents, compositions and substances of the present ~~invention~~disclosure may include as active ingredients APCs or exosomes that present any of the present peptides.

[0013] The peptides or polynucleotides of the present ~~invention~~disclosure may be used to induce APCs that present on the surface a complex of an HLA antigen and a present peptide, for example, by contacting APCs derived from a subject with the peptide or introducing a polynucleotide encoding a peptide of the present ~~invention~~disclosure into APCs. Such APCs have high CTL inducibility against target peptides and find use in cancer immunotherapy. Accordingly, the present ~~invention~~disclosure encompasses the methods for inducing APCs with CTL inducibility as well as APCs obtained by such methods. In another aspect, there is provided a method for inducing an antigen-presenting cell (APC) with CTL inducibility comprising contacting an APC with the peptide as defined above in vitro or ex vivo.

[0014] It is a further object of the present ~~invention~~disclosure to provide a method for inducing CTL, such methods including the step of co-culturing CD8 positive cells with APCs or exosomes presenting the peptide of the present ~~invention~~disclosure on its surface or the step of introducing a gene that includes a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide binding to the present peptide.

In another aspect, there is provided a method for inducing CTL by a method that comprises a step selected from the group consisting of:

(a) co-culturing CD8 positive T cells with APCs that present on the surface a complex of an HLA antigen and the peptide as defined above; and

(b) co-culturing CD8 positive T cells with exosomes that present on the surface a complex of an HLA antigen and the peptide as defined above.

CTLs obtained by such methods 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, CML, colorectal cancer, esophageal cancer, NSCLC, lymphoma, pancreatic cancer, prostate cancer, renal carcinoma and SCLC. Therefore, it is yet another object of the present ~~invention~~disclosure to provide CTLs obtained by the present methods.

[0015] It is yet another object of the present ~~invention~~disclosure to provide methods for inducing an

immune response against cancer in a subject in need thereof, such methods including the step of administering compositions or substances including the ECT2 polypeptides or immunologically active fragments thereof, polynucleotides encoding ECT2 polypeptides, exosomes or the APCs presenting ECT2 polypeptides.

The applicability of the present ~~invention~~disclosure extends to any of a number of diseases relating to or arising from ECT2 overexpression, such as cancer, examples of which include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophageal cancer, NSCLC, lymphoma, pancreatic cancer, prostate cancer, renal carcinoma and SCLC.

[0016] More specifically, the present ~~invention~~disclosure provides followings:

[1] An isolated peptide consisting of the amino acid sequence of SEQ ID NO: 42 or an immunologically active fragment thereof, wherein said peptide binds an HLA antigen and induces cytotoxic T lymphocytes (CTLs),

[2] The isolated peptide of [1], wherein the HLA antigen is HLA-A2,

[3] The isolated peptide of [1] or [2], wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 40,

[4] An isolated peptide selected from the group consisting of:

(a) an isolated peptide that binds to an HLA antigen and induces cytotoxic T lymphocytes (CTL)and consists of the amino acid sequence of SEQ ID NO: 42 or an immunologically active fragment thereof,

(b) the isolated peptide of (a), wherein the HLA antigen is HLA-A2,

(c) the isolated peptide of (a) or (b), which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 40, and

(d) the isolated peptide of (a) or (b) consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 40, wherein 1, 2, or several amino acid(s) are substituted, deleted, inserted or added, provided said modified peptide retains the CTL inducibility of the original peptide,

[5] The isolated peptide of [4], which consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 40, wherein the peptide has one or both of the following characteristics:

(a) the second amino acid from the N-terminus is selected from the group consisting of leucine or methionine; and

(b) the C-terminal amino acid is selected from the group consisting of valine or leucine,

[6] The isolated peptide of any one of [1] to [5], wherein said peptide is nonapeptide or decapeptide,

[7] An isolated polynucleotide encoding the peptide of any one of [1] to [6],

[8] A composition for inducing CTL, wherein the composition comprises one or

more peptide(s) of any one of [1] to [6], or one or more polynucleotide(s) of [7],
[9] A pharmaceutical composition for the treatment and/or prophylaxis and/or prevention of postoperative recurrence of cancer, wherein the composition comprises one or more peptide(s) of any one of [1] to [6], or one or more polynucleotides of [7],
[10] The pharmaceutical composition of [9], wherein said composition is formulated for the administration to a subject whose HLA antigen is HLA-A2,
[11] The pharmaceutical composition of [9] or [10], wherein said composition is formulated for the treatment of cancer,
[12] A method for inducing an antigen-presenting cell (APC) with CTL inducibility comprising a step selected from the group consisting of:
(a) contacting an APC with a peptide of any one of [1] to [6] in vitro, ex vivo or in vivo, and
(b) introducing a polynucleotide encoding the peptide of any one of [1] to [6] into an APC,
[13] A method for inducing CTL by a method that comprises a step selected from the group consisting of:
(a) co-culturing CD8 positive T cells with APCs that present on the surface a complex of an HLA antigen and the peptide of any one of [1] to [6];
(b) co-culturing CD8 positive T cells with exosomes that present on the surface a complex of an HLA antigen and a peptide of any one of [1] to [6]; and
(c) introducing a gene that comprises a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide bound to a peptide of any one of [1] to [6] into a T cell,
[14] An isolated APC that presents on its surface a complex of an HLA antigen and the peptide of any one of [1] to [6],
[15] The APC of [14], which is induced by the method of [12],
[16] An isolated CTL that targets any of the peptides of [1] to [6],
[17] A CTL of [16], induced by the method of [13],
[18] A method of inducing an immune response against cancer in a subject in need thereof, said method comprising the step of administering to the subject a composition comprising a peptide of [1] to [6], an immunologically active fragment thereof, or a polynucleotide encoding the peptide or the fragment,
[19]An antibody or immunologically active fragment thereof against any of the peptides of [1] to [6],
[20]A vector comprising a nucleotide sequence encoding any of the peptides of [1] to [6],
[21]A diagnostic kit comprising any of the peptides of [1] to [6], the nucleotide of [7] or the antibody of [19].

[0017] It is to be understood that both the foregoing summary of the present ~~invention~~disclosure and

the following detailed description are of exemplified embodiments, and not restrictive of the present ~~invention~~disclosure or other alternate embodiments of the present ~~invention~~disclosure.

[0018] In addition to the above, other objects and features of the ~~invention~~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 ~~invention~~present disclosure and the following detailed description are of exemplified embodiments, and not restrictive of the ~~invention~~present disclosure or other alternate embodiments of the ~~invention~~present disclosure. In particular, while the ~~invention~~present disclosure is described herein with reference to a number of specific embodiments, it will be appreciated that the description is illustrative of the ~~invention~~present disclosure and is not constructed as limiting of the ~~invention~~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 ~~invention~~present disclosure, as described by the appended claims. Likewise, other objects, features, benefits and advantages of the present ~~invention~~disclosure will be apparent from this summary and certain embodiments 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 Drawings

[0019] Various aspects and applications of the present invention will become apparent to the skilled artisan upon consideration of the brief description of the figures and the detailed description of the present invention and its preferred embodiments which follows.

[0020] [fig. 1] Figure 1 is composed of a series of photographs, (a) to (c), depicting the results of IFN-gamma ELISPOT assays on CTLs that were induced with peptides derived from ECT2. The CTLs in well number #6 stimulated with ECT2-A02-9-34 (SEQ ID NO: 1) (a), in well number #5 stimulated with ECT2-A02-9-664 (SEQ ID NO: 3) (b) and in well number #1 stimulated with ECT2-A02-10-33 (SEQ ID NO: 21) (c) showed potent IFN-gamma production as compared with the control. The square on the well of these pictures indicates that the cells from corresponding well were expanded to establish CTL lines. In the figures, "+" indicates the IFN-gamma production against target cells pulsed with the appropriate peptide, and "-" indicates the IFN-gamma production against target cells not pulsed with any peptides.

[0021] [fig. 2] Figure 2 is composed of a series of line graphs, (a) to (c), depicting the results of an IFN-gamma ELISA assay demonstrating the IFN-gamma production of CTL lines stimulated with (a) ECT2-A02-9-34 (SEQ ID NO: 1), (b) ECT2-A02-9-664 (SEQ ID NO: 3) and (c) ECT2-A02-10-33 (SEQ ID NO: 21). The results demonstrate that CTL

Claims

[Claim 1] An isolated peptide consisting of the amino acid sequence of SEQ ID NO: 42 or an immunologically active fragment thereof, wherein said peptide binds an HLA antigen and induces cytotoxic T lymphocytes (CTLs).

[Claim 2] The isolated peptide of claim 1, wherein the HLA antigen is HLA-A2.

[Claim 3] The isolated peptide of claim 1 or 2, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 40.

[Claim 4] An isolated peptide selected from the group consisting of:
(a) an isolated peptide that binds to an HLA antigen and induces cytotoxic T lymphocytes (CTL)and consists of the amino acid sequence of SEQ ID NO: 42 or an immunologically active fragment thereof,
(b) the isolated peptide of (a), wherein the HLA antigen is HLA-A2,
(c) the isolated peptide of (a) or (b), which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 40, and
(d) the isolated peptide of (a) or (b) consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 40, wherein 1, 2, or several amino acid(s) are substituted, deleted, inserted or added, provided said modified peptide retains the CTL inducibility of the original peptide.

[Claim 5] The isolated peptide of claim 4, which consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 40, wherein the peptide has one or both of the following characteristics:
(a) the second amino acid from the N-terminus is selected from the group consisting of leucine or methionine; and
(b) the C-terminal amino acid is selected from the group consisting of valine or leucine.

[Claim 6] The isolated peptide of any one of claims 1 to 5, wherein said peptide is nonapeptide or decapeptide.

[Claim 7] An isolated polynucleotide encoding the peptide of any one of claims 1 to 6.

[Claim 8] A composition for inducing CTL, wherein the composition comprises one or more peptide(s) of any one of claims 1 to 6, or one or more polynucleotide(s) of claim 7.

[Claim 9] A pharmaceutical composition for the treatment and/or prophylaxis

and/or prevention of postoperative recurrence of cancer, wherein the composition comprises one or more peptide(s) of any one of claims 1 to 6, or one or more polynucleotides of claim 7.

[Claim 10] The pharmaceutical composition of claim 9, wherein said composition is formulated for the administration to a subject whose HLA antigen is HLA-A2.

[Claim 11] The pharmaceutical composition of claim 9 or 10, wherein said composition is formulated for the treatment of cancer.

[Claim 12] A method for inducing an antigen-presenting cell (APC) with CTL inducibility comprising a step selected from the group consisting of:
(a) contacting an APC with a peptide of any one of claims 1 to 6 in vitro, .ex vivo or in vivo, and
(b) introducing a polynucleotide encoding the peptide of any one of claims 1 to 6 into an APC.

[Claim 13] A method for inducing CTL by a method that comprises a step selected from the group consisting of:
(a) co-culturing CD8 positive T cells with APCs that present on the surface a complex of an HLA antigen and the peptide of any one of claims 1 to 6;
(b) co-culturing CD8 positive T cells with exosomes that present on the surface a complex of an HLA antigen and a peptide of any one of claims 1 to 6; and
(c) introducing a gene that comprises a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide bound to a peptide of any one of claims 1 to 6 into a T cell.

[Claim 14] An isolated APC that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 6.

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

[Claim 16] An isolated CTL that targets any of the peptides of claims 1 to 6.

[Claim 17] A CTL of claim 16, induced by the method of claim 13.

[Claim 18] A method of inducing an immune response against cancer in a subject in need thereof, said method comprising the step of administering to the subject a composition comprising a peptide of claims 1 to 6, an immunologically active fragment thereof, or a polynucleotide encoding the peptide or the fragment.

[Claim 19] An antibody or immunologically active fragment thereof against any of the peptides of claims 1 to 6.

[Claim 20] A vector comprising a nucleotide sequence encoding any of the

peptides of claims 1 to 6.

[Claim 21] A diagnostic kit comprising any of the peptides of claims 1 to 6, the nucleotide of claim 7 or the antibody of claim 19.

[Claim 22] The isolated peptide of any one of claims 1 to 6 or the peptide encoded by polynucleotide of claim 7, wherein the peptide consists of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3 and 21.

The invention claimed is:

1. An isolated peptide of less than 15 amino acids having cytotoxic T lymphocyte (CTL) inducibility selected from the group consisting of:

   (a) an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3 and 21, and

   (b) an isolated peptide having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3 and 21, in which 1 or 2 amino acid(s) are substituted or added.

2. The isolated 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: 1, 3 or 21 is substituted with methionine; and

   (b) the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 1, 3 or 21 is substituted with an amino acid selected from the group consisting of valine and leucine.

3. The isolated peptide of claim 1, wherein said peptide is nonapeptide or decapeptide.

4. A composition for inducing CTL, wherein the composition comprises one or more peptide(s) of claim 1.

5. A pharmaceutical composition for the treatment of cancer over expressing ECT2, wherein the composition comprises one or more peptide(s) of claim 1.

6. The pharmaceutical composition of claim 5, wherein said pharmaceutical composition is formulated for the administration to a subject whose HLA antigen is HLA-A2.

7. A method for inducing an antigen-presenting cell (APC) with CTL inducibility comprising contacting an APC with the peptide of claim 1 in vitro or ex vivo.

8. A method for inducing CTL by a method that comprises a step selected from the group consisting of:

   (a) co-culturing CD8 positive T cells with APCs that present on the surface a complex of an HLA antigen and the peptide of claim 1; and

   (b) co-culturing CD8 positive T cells with exosomes that present on the surface a complex of an HLA antigen and the peptide of claim 1.

9. A kit comprising any of the peptides of claim 1.

10. The isolated peptide of claim 1, wherein the peptide consists of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3 and 21.

11. The composition of claim 4, wherein the composition comprises a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier is sterilized water, physiological saline or phosphate buffer.

12. The pharmaceutical composition of claim 5, wherein the pharmaceutical composition comprises a pharmaceutically acceptable carrier, wherein the pharmaceutically acceptable carrier is sterilized water, physiological saline or phosphate buffer.

13. The pharmaceutical composition of claim 5, wherein the pharmaceutical composition comprises Incomplete Freund's adjuvant as an adjuvant.