Summary ~~of Invention~~

[0008] The present ~~invention~~disclosure is based, in part, on the discovery of novel peptides that may serve as suitable targets of immunotherapy. Because TAAs are generally perceived for the immune system as "self" and therefore often have no immunogenicity, the discovery of appropriate targets is of extreme importance. Recognizing that MELK (as described, for example, in SEQ ID NO: 47) (encoded by the gene of GenBank Accession No. NM_014791 (SEQ ID NO: 46)) has been identified as up-regulated in tissues of endometriosis and cancers including but not limited to breast cancer, bladder cancer, cervical cancer, cholangiocellular carcinoma, chronic myeloid leukemia (CML), colorectal cancer, , esophagus cancer, gastric cancer, liver cancer, non-small cell lung cancer (NSCLC), lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma and small cell lung cancer (SCLC) (WO2010/013485), the present ~~invention~~disclosure focuses on MELK as a target of cancer immunotherapy.

[0009] To that end, the present ~~invention~~disclosure is directed at least in part, to the identification of specific modified epitope peptides of MELK that possess the ability to induce cytotoxic T lymphocytes (CTLs) specific to MELK. As discussed in detail below, peripheral blood mononuclear cells (PBMCs) obtained from healthy donors were stimulated using A*2402 binding candidate peptides derived from modified MELK epitope peptide, i.e., wild type MELK-A24-9-87_WT (SEQ ID NO: 6). CTL lines with specific cytotoxicity against HLA-A24 positive target cells pulsed with each of candidate peptides were then established. Taken together, these results demonstrate that these peptides are HLA-A24 restricted epitope peptides that can induce potent and specific immune responses against cells expressing MELK. The results further demonstrate that MELK is strongly immunogenic and that the epitopes thereof are effective targets for tumor immunotherapy.

[0010] Accordingly, it is an object of the present ~~invention~~disclosure to provide isolated peptides of the modified epitope peptide derived from MELK (SEQ ID NO: 47), specifically the modified epitope peptide of wild type MELK-A24-9-87_WT (SEQ ID NO: 6), or immunologically active fragments thereof that bind to HLA antigens. The present peptides have CTL inducibility. Thus, they can be used to induce CTL ex vivo or can be administered to a subject for inducing immune responses against endometriosis and cancers, examples of which include, but are not limited to, breast cancer, bladder cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, , esophagus cancer, gastric cancer, liver cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma and SCLC. Preferred peptides are nonapeptides, and typically, consist of the amino acid sequence

selected from the group consisting of SEQ ID NOs: 35-45. Of these, the peptides having an amino sequence selected from among SEQ ID NOs: 35, 41 and 44 showed particularly strong CTL inducibility and thus are particularly useful in the present ~~invention~~disclosure.

The present ~~invention~~disclosure also contemplates modified peptides, having an amino acid sequence of SEQ ID NOs: 35-45, wherein one, two or more amino acid(s) is/are substituted, deleted or added, so long as the modified peptides retain the requisite original CTL inducibility of the original peptide.

In an aspect, there is provided an isolated peptide that can bind to an HLA antigen and has cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide consists of an amino acid sequence having one or more amino acid substitutions in the amino acid sequence of SEQ ID NO: 6 selected from the group consisting of (a) to (c): (a) the N-terminal amino acid, (b) the third amino acid from the N-terminus and (c) the third amino acid from the C-terminus.

In another aspect, there is provided an isolated peptide that can bind to an HLA antigen and has cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 44, 35, and 41, in which 1 or 2 amino acid(s) are inserted, substituted, deleted or added, wherein said peptide is not SEQ ID NO: 6.

In another aspect, there is provided a composition for inducing a CTL, wherein the composition comprises one or more peptide(s) as defined above.

In another aspect, there is provided a composition comprising one or more peptide(s) as defined above.

[0011] Further, the present ~~invention~~disclosure provides isolated polynucleotides encoding any of the peptides of the present ~~invention~~disclosure. These polynucleotides can be used to induce antigen-expressing cells (APCs) with CTL inducibility, like the peptides of the present ~~invention~~disclosure, or can be administered to a subject for inducing immune responses against cancers.

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, it is an object of the present ~~invention~~disclosure to provide substances that induce CTLs, such substances including one or more peptides of the present ~~invention~~disclosure or polynucleotides encoding such peptides. The present ~~invention~~disclosure further contemplates pharmaceutical substances including one or more of the peptides of the present ~~invention~~disclosure or polynucleotides encoding such peptides, such compositions can be used for treating and/or for the prophylaxis of endometriosis and cancers, such cancers including, but not limited, breast cancer, bladder cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, , esophagus cancer, gastric cancer, liver cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma and SCLC, and/or for preventing postoperative recurrence thereof. Thus, it is yet another object of the present ~~invention~~disclosure to provide pharmaceutical composition or substances formulated for the treatment and/or prevention of endometriosis or cancer, and/or prevention of postoperative recurrence thereof and including any of the peptides or polynucleotides of the present ~~invention~~disclosure. Instead of or in addition to the present peptides or polynucleotides, the present substances or pharmaceutical of the present ~~invention~~disclosure substances may optionally include, as the active ingredient, APCs or exosomes which present any of the peptides of the present ~~invention~~disclosure.

[0012] The peptides and 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 present peptide or introducing a polynucleotide encoding the present peptide into APCs. Such APCs have high CTL inducibility against the target peptides and thus are useful for cancer immunotherapy. Accordingly, it is another object of the present ~~invention~~disclosure to provide

methods for inducing APCs with CTL inducibility as well as APCs obtained by such methods.

In another aspect, there is provided an *in vitro* method for inducing an antigen-presenting cell (APC) with CTL inducibility, wherein the method comprises the step of contacting an APC with the peptide as defined above in vitro.

[0013] It is a further object of the present ~~invention~~disclosure to provide methods for inducing CTL, methods that includes the step of co-culturing CD8-positive cells with APCs or exosomes presenting a 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 binding to the present peptide. CTLs obtainable by the present methods also find utility in the treatment and/or preventing diseases in which MELK is overexpressed, such as endometriosis, breast cancer, bladder cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophagus cancer, gastric cancer, liver cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma and SCLC, but are not limited to. Therefore, it is another object of the present ~~invention~~disclosure to provide CTLs obtained by the present methods.

In another aspect, there is provided an *in vitro* method for inducing a CTL by any of the methods comprising at least one of the following steps:

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

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

[0014] Moreover, a further object of the present ~~invention~~disclosure is to provide methods for inducing an immune response against cancer in a subject in need thereof, such methods including the step of administering to the subject a substance or a composition containing modified MELK or immunologically active fragments thereof, polynucleotides encoding modified MELK or the fragments thereof, and exosomes or APCs presenting modified MELK or the fragments thereof.

The applicability of the present ~~invention~~disclosure extends to any of a number of diseases relating to or arising from MELK overexpression including endometriosis and cancer, examples of which include, but are not limited to, breast cancer, bladder cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophagus cancer, gastric cancer, liver cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma and SCLC.

[0015] More specifically, the present ~~invention~~disclosure provides followings:

[1] An isolated peptide binding to HLA antigen and having cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide consists of the amino acid sequence of SEQ ID NO: 6 or consists of an amino acid sequence comprising one or more amino acid substitutions in the amino acid sequence of SEQ ID NO: 6,

[2] The isolated peptide of [1], wherein the HLA antigen is HLA-A24,

[3] The isolated peptide of [1], wherein the polypeptide comprises one or more amino acid substitutions at the positions selected from the group consisting of (a) - (d) in the amino acid sequence of SEQ ID NO: 6:

(a) N-terminal amino acid,

(b) third amino acid from the N-terminus,

(c) third amino acid from the C-terminus and

for the administration to a subject whose HLA antigen is HLA-A24,
[16] The pharmaceutical composition of [14] or [15], wherein said composition is formulated for treating cancer or endometriosis,
[17] A method for inducing an antigen-presenting cell (APC) with CTL inducibility, wherein the method comprises one of the following steps:
(a) contacting an APC with a peptide of any one of [1] to [11] in vitro, ex vivo or in vivo; and
(b) introducing a polynucleotide encoding a peptide of any one of [1] to [9] into an APC,
[18] A method for inducing CTL by any of the methods comprising at least one of the following steps:
(a) co-culturing CD8-positive T cells with APCs, which presents on its surface a complex of an HLA antigen and a peptide of any one of [1] to [11];
(b) co-culturing CD8-positive T cells with exosomes, which presents on its surface a complex of an HLA antigen and a peptide of any one of [1] to [11]; and
(c) introducing a gene that comprises a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide binding to a peptide of any one of [1] to [9] into a T cell,
[19] An isolated APC that presents on its surface a complex of an HLA antigen and a peptide of any one of [1] to [11],
[20] The APC of [19], wherein said APC is induced by the method of [17],
[21] An isolated CTL that targets a peptide of any one of [1] to [11],
[22] The CTL of [21], which is induced by the method of [18], and
[23] A method of inducing immune response against cancer or endometriosis in a subject comprising administering to the subject a composition comprising a peptide of any one of [1] to [11], an immunologically active fragment thereof, or a polynucleotide encoding the peptide or the fragment.

[0016] 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 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

[0017] 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.

[0018] [fig.1]Fig. 1 depicts the photographs showing the result of IFN-gamma ELISPOT assays on CTLs of donor A induced with peptides derived from MELK. The CTLs stimulated with MELK-A24-9-87 (SEQ ID NO: 6) (a) MELK-A24-10-637 (SEQ ID NO: 23) (b) MELK-A24-9-199 (SEQ ID NO: 1) (d) and MELK-A24-9-78 (SEQ ID NO: 21) (e) showed potential IFN-gamma productive ability. In contrast, as typical case of negative data, specific IFN-gamma production was not shown from the CTLs stimulated with MELK-A24-9-96 (SEQ ID NO: 2) (c). In the figures, "+" indicates the IFN-gamma production against the target cells pulsed with cognate peptides, and "-" indicates the IFN-gamma production against target cells not pulsed with any peptides.

[0019] [fig.2]Fig. 2 depicts the line graphs showing the result of the establishment of CTL lines. The potent IFN-gamma production was detected from the CTL lines stimulated with MELK-A24-9-87 (SEQ ID NO: 6) (a), MELK-A24-10-637 (SEQ ID NO: 23) (b) and MELK-A24-9-199 (SEQ ID NO: 1) (c) by IFN-gamma ELISA assay. In the figures, "black lozenge" indicates the IFN-gamma production against target cells pulsed with cognate peptides and "white square" indicates the IFN-gamma production against target cells not pulsed with any peptides.

[0020] [fig.3]Fig. 3 depicts the line graph showing the result of the establishment of the CTL clone. The potent IFN-gamma production was detected from the CTL clone stimulate with MELK-A24-9-87 (SEQ ID NO: 6) (a) and MELK-A24-9-199 (SEQ ID NO: 1) (b) by IFN-gamma ELISA assay. In the figure, "black lozenge" indicates the IFN-gamma production against target cells pulsed with MELK-A24-9-87 (SEQ ID NO: 6) and "white square" indicates the IFN-gamma production against target cells not pulsed with any peptides.

[0021] [fig.4]Fig. 4 depicts the line graph showing the specific CTL activity against the target cells that exogenously express MELK and HLA-A*2402. COS7 cells transfected with

Claims

[Claim 1] An isolated peptide binding to HLA antigen and having cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide consists of the amino acid sequence of SEQ ID NO: 6 or consists of an amino acid sequence comprising one or more amino acid substitutions in the amino acid sequence of SEQ ID NO: 6.

[Claim 2] The isolated peptide of claim 1, wherein the HLA antigen is HLA-A24.

[Claim 3] The isolated peptide of claim 1, wherein the polypeptide comprises one or more amino acid substitutions at the positions selected from the group consisting of (a) - (d) in the amino acid sequence of SEQ ID NO: 6:
(a) N-terminal amino acid,
(b) third amino acid from the N-terminus,
(c) third amino acid from the C-terminus and
(d) C-terminal amino acid.

[Claim 4] The isolated peptide of claim 3, wherein the polypeptide comprises one or more amino acid substitutions selected from the group consisting of (i) to (iv):
(i) amino acid substitution from E to K or R at the N-terminal amino acid in the amino acid sequence of SEQ ID NO: 6,
(ii) amino acid substitution from C to E, I, L, M, N or P at the third amino acid from the N-terminus in the amino acid sequence of SEQ ID NO: 6,
(iii) amino acid substitution from E to N or Q at the third amino acid from the C-terminus in the amino acid sequence of SEQ ID NO: 6 and
(iv) amino acid substitution from F to L at the C-terminal amino acid in the amino acid sequence of SEQ ID NO: 6.

[Claim 5] The isolated peptide of claim 4, wherein the peptide comprises a single amino acid substitution.

[Claim 6] The isolated peptide of claim 4, wherein the peptide comprises two amino acid substitutions.

[Claim 7] The isolated peptide of claim 4, wherein the peptide comprises three amino acid substitutions.

[Claim 8] The isolated peptide of claim 4, wherein the peptide comprises four amino acid substitutions.

[Claim 9] The isolated peptide of claim 4-5, which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 35-45.

[Claim 10] An isolated peptide binding to HLA antigen and having cytotoxic T lymphocyte (CTL) inducibility, wherein the said peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 35-45, wherein 1, 2, or several amino acid(s) are inserted, substituted, deleted or added.

[Claim 11] The peptide of claim 10 having one or both of the following characteristics:
(a) the second amino acid from the N-terminus is selected from the group of phenylalanine, tyrosine, methionine and tryptophan; and
(b) the C-terminal amino acid is selected from the group of phenylalanine, leucine, isoleucine, tryptophan and methionine.

[Claim 12] An isolated polynucleotide encoding a peptide of any one of claims 1 to 11.

[Claim 13] A substance for inducing CTL, wherein the substance comprises one or more peptide(s) of any one of claims 1 to 11, or one or more polynucleotide(s) of claim 12.

[Claim 14] A pharmaceutical composition for the treatment and/or prophylaxis of cancers or endometriosis, and/or the prevention of postoperative recurrence thereof, wherein the composition comprises one or more peptide(s) of any one of claims 1 to 11, or one or more polynucleotide(s) of claim 12.

[Claim 15] The pharmaceutical composition of claim 14, wherein said composition is formulated for the administration to a subject whose HLA antigen is HLA-A24.

[Claim 16] The pharmaceutical composition of claim 14 or 15, wherein said composition is formulated for treating cancer or endometriosis.

[Claim 17] A method for inducing an antigen-presenting cell (APC) with CTL inducibility, wherein the method comprises one of the following steps:
(a) contacting an APC with a peptide of any one of claims 1 to 11 in vitro, ex vivo or in vivo; and
(b) introducing a polynucleotide encoding a peptide of any one of claims 1 to 9 into an APC.

[Claim 18] A method for inducing CTL by any of the methods comprising at least one of the following steps:
(a) co-culturing CD8-positive T cells with APCs, which presents on its surface a complex of an HLA antigen and a peptide of any one of claims 1 to 11;
(b) co-culturing CD8-positive T cells with exosomes, which presents

on its surface a complex of an HLA antigen and a peptide of any one of claims 1 to 11; and

(c) introducing a gene that comprises a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide binding to a peptide of any one of claims 1 to 9 into a T cell.

[Claim 19] An isolated APC that presents on its surface a complex of an HLA antigen and a peptide of any one of claims 1 to 11.

[Claim 20] The APC of claim 19, wherein said APC is induced by the method of claim 17.

[Claim 21] An isolated CTL that targets a peptide of any one of claims 1 to 11.

[Claim 22] The CTL of claims 21, which is induced by the method of claim 18.

[Claim 23] A method of inducing immune response against cancer or endometriosis in a subject comprising administering to the subject a composition comprising a peptide of any one of claims 1 to 11, an immunologically active fragment thereof, or a polynucleotide encoding the peptide or the fragment.

Claims

[Claim 1] An isolated peptide that can bind to an HLA antigen and has cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide consists of an amino acid sequence having one or more amino acid substitutions in the amino acid sequence of SEQ ID NO: 6 selected from the group consisting of (a) to (c): (a) the N-terminal amino acid, (b) the third amino acid from the N-terminus and (c) the third amino acid from the C-terminus.

[Claim 2] The isolated peptide of claim 1, wherein the HLA antigen is HLA-A24.

[Claim 3] The isolated peptide of claim 1, wherein the peptide has one or more amino acid substitutions selected from the group consisting of (i) to (iii):

(i) amino acid substitution from E to K or R at the N-terminal amino acid in the amino acid sequence of SEQ ID NO: 6,

(ii) amino acid substitution from C to E, I, L, M, N or P at the third amino acid from the N-terminus in the amino acid sequence of SEQ ID NO: 6, and

(iii) amino acid substitution from E to N or Q at the third amino acid from the C-terminus in the amino acid sequence of SEQ ID NO: 6.

[Claim 4] The isolated peptide of claim 3, wherein the number of the amino acid substitutions is one.

[Claim 5] The isolated peptide of claim 3, wherein the number of the amino acid substitutions is two or three.

[Claim 6] The isolated peptide of claim 4, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 44, 35, and 41.

[Claim 7] An isolated peptide that can bind to an HLA antigen and has cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 44, 35, and 41, in which 1 or 2 amino acid(s) are inserted, substituted, deleted or added, wherein said peptide is not SEQ ID NO: 6.

[Claim 8] The isolated peptide of claim 7, 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: 44, 35 or 41 is substituted with an amino acid selected from the group consisting of phenylalanine, methionine and tryptophan; and

(b) the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 44, 35 or 41 is substituted with an amino acid selected from the group consisting of, leucine, isoleucine, tryptophan and methionine.

[Claim 9] A composition for inducing a CTL, wherein the composition comprises one or more peptide(s) of claim 1 or 7.

[Claim 10] A composition comprising one or more peptide(s) of claim 1 or 7.

[Claim 11] The composition of claim 10, wherein said composition is formulated for the administration to a subject whose HLA antigen is HLA-A24.

[Claim 12] An *in vitro* method for inducing an antigen-presenting cell (APC) with CTL inducibility, wherein the method comprises the step of

contacting an APC with the peptide of claim 1 or 7 in vitro.

[Claim 13] An *in vitro* method for inducing a CTL by any of the methods comprising at least one of the following steps:

(a) co-culturing CD8-positive T cells with APCs which present on its surface a complex of an HLA antigen and the peptide of claim 1 or 7 and

(b) co-culturing CD8-positive T cells with exosomes which present on its surface a complex of an HLA antigen and the peptide of claim 1 or 7.