

pp.1838-1846). However, it is difficult to estimate if a predicted epitope peptide can be processed naturally in the target cells and expressed on the target cell surface with HLA molecule. Moreover, the algorithms, for example BIMAS (http://bimas.dcrt.nih.gov/cgi-bin/molbio/ken_parker_comboform) (Parker KC, et al., (1994) *J Immunol.*;152(1):163-75.; Kuzushima K, et al., (2001) *Blood.*;98(6):1872-81.)) can suggest less than rigorous HLA molecule-binding peptides (Bachinsky MM, et. al., *Cancer Immun.* 2005 Mar 22;5:6.). Thus, identifying epitope peptides remains challenging and difficult.

- [0006] MELK, maternal embryonic leucine zipper kinase, was previously identified as a new member of the snf1/AMPK serine-threonine kinase family that is involved in mammalian embryonic development (Heyer BS et al., *Dev Dyn.* 1999 Aug 215(4):344-51). The gene was shown to play an important role in stem cell renewal (Nakano I et al., *J Cell Biol.* 2005 Aug 1, 170(3):413-27), cell-cycle progression (Blot J et al., *Dev Biol.* 2002 Jan 15, 241(2):327-38; Seong HA et al., *Biochem J.* 2002 Feb 1, 361(Pt 3):597-604) and pre-mRNA splicing (Vulsteke V et al., *J Biol Chem.* 2004 Mar 5, 279(10):8642-7. Epub 2003 Dec 29). In addition, through gene expression profile analysis using a genome-wide cDNA microarray containing 23,040 genes, MELK was recently shown to be up-regulated in breast cancer (Lin ML et al., *Breast Cancer Res.* 2007; 9 (1):R17, WO2006/016525, WO2008/023841). In fact, MELK is up-regulated in several cancer cells, for example lung, bladder, lymphoma and cervical cancer cells (See WO2004/031413, WO2007/013665, and WO2006/085684, the disclosures of which are incorporated by reference herein). Northern blot analysis on multiple human tissues and cancer cell lines demonstrated that MELK was over-expressed at a significantly high level in a great majority of breast cancers and cell lines, but was not expressed in normal vital organs (heart, liver, lung and kidney) (WO2006/016525). Furthermore, suppression of MELK expression by siRNA was shown to significantly inhibit growth of human breast cancer cells. Accordingly, MELK is considered to be a suitable target for cancer immunotherapy and epitope peptides derived therefrom may be expected to serve as cancer immunotherapeutics effective in the treatment of a wide array of cancer types.

Summary of Invention-

- [0007] The present ~~invention~~disclosure is based in part on the discovery of 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 of extreme importance. Recognizing that MELK has been identified as up-regulated in cancer tissues of breast cancer, bladder cancer, cervical cancer, cholangio-cellular carcinoma, chronic myeloid leukemia (CML), colorectal cancer, en-

dometriosis, 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 (SCC), the present ~~invention~~disclosure targets this maternal embryonic leucine zipper kinase (MELK) protein (SEQ ID NO: 94) encoded by the gene of GenBank Accession No. NM_014791 (SEQ ID NO: 93) for further analysis. In particular, MELK gene products containing epitope peptides that elicit CTLs specific to the corresponding molecules were selected for study. Peripheral blood mononuclear cells (PBMCs) obtained from a healthy donor were stimulated using HLA-A*2402 or HLA-A*0201 binding candidate peptides derived from MELK. CTLs that specifically recognize HLA-A24 or HLA-A02 positive target cells pulsed with the respective candidate peptides were established, and HLA-A24 or HLA-A02 restricted epitope peptides that can induce potent and specific immune responses against MELK expressed on the surface of tumor cells were identified. These results demonstrate that MELK is strongly immunogenic and the epitopes thereof are effective targets for tumor immunotherapy.

[0007a] In one aspect, there is provided an isolated peptide of less than 15 amino acids, wherein said peptide comprises an amino acid sequence selected from the group consisting of:

- (a) SEQ ID NOs:36, 46, 57, 60 and 62; and
- (b) SEQ ID NOs:36, 46, 57, 60 and 62, in which 1 or 2 amino acids are substituted, inserted, deleted and/or added, and has cytotoxic T lymphocyte (CTL) inducibility.

[0008] Accordingly, it is an object of the present ~~invention~~disclosure to provide peptides having CTL inducibility as well as an amino acid sequence selected from the group of SEQ ID NOs: 14, 21, 23, 27, 36, 46, 57, 60 and 62. In addition, the present ~~invention~~disclosure contemplates modified peptides, having an amino acid sequence of SEQ ID NOs: 14, 21, 23, 27, 36, 46, 57, 60 or 62, wherein one, two or more amino acids are substituted, deleted, incorporated, and/or added, so long as the modified peptides retain the original CTL inducibility. In another aspect, there is provided an isolated polynucleotide encoding a peptide as described herein.

[0008a] In another aspect, there is provided an *in vitro* method for inducing an antigen-presenting cell with high CTL inducibility, wherein the method comprises the step of contacting an antigen-presenting cell with a peptide as described herein, or the step of introducing a polynucleotide encoding the peptide in an expressible form into an antigen-presenting cell. In another aspect, there is provided an *in vitro* method for inducing CTL by using a peptide as described herein.

[0008b] In another aspect, there is provided an isolated CTL which targets any of the peptides as described herein. In another aspect, there is provided an isolated CTL which is induced by a peptide as described herein.

[0008c] In another aspect, there is provided an isolated antigen-presenting cell that presents a complex formed between a HLA antigen and a peptide as described herein on its surface.

[0009] When administered to a subject, the present peptides are presented on the surface of antigen-expressing cells and then induce CTLs targeting the respective peptides. Therefore, it is an object of the present invention to provide antigen-presenting cells which present any of the present peptides, as well as methods for inducing antigen-presenting cells. An anti-tumor immune response is induced by the administration of the present MELK polypeptides or polynucleotide encoding the polypeptides, as well as exosomes and antigen-presenting cells which present the MELK polypeptides. Therefore, it is yet another object of the present invention to provide pharmaceutical agents or compositions containing the polypeptides or polynucleotides encoding them, as well as the exosomes and antigen-presenting cells as their active ingredients. The pharmaceutical agents of the present invention find use as vaccines.

[0009a] In another aspect, there is provided a pharmaceutical composition for the treatment and/or prophylaxis of cancer, and/or the prevention of a postoperative recurrence thereof, wherein the pharmaceutical composition comprises an active ingredient selected from the group consisting of:

- (a) one or more peptides as described herein;
- (b) one or more polynucleotides encoding the peptide in an expressible form;
- (c) one or more antigen-presenting cells and/or exosomes, which antigen-presenting cells and exosomes present a complex formed between a HLA antigen and a peptide as described herein on its surface;
- (d) one or more CTLs induced against a peptide as described herein; and
- (e) combinations thereof.

[0009b] In another aspect, there is provided a composition for inducing an immune response against a cancer in a subject, wherein said composition comprises an active ingredient selected from the group consisting of:

- (a) one or more peptides as described herein;
- (b) one or more polynucleotides encoding the peptide in an expressible form;

(c) one or more antigen-presenting cells and/or exosomes, which antigen-presenting cells and exosomes present a complex formed between a HLA antigen and a peptide as described herein on its surface;

(d) one or more CTLs induced against a peptide as described herein; and

(e) combinations thereof.

[0009c] In another aspect, there is provided a composition for inducing a CTL, wherein said composition comprises an active ingredient selected from the group consisting of:

(a) one or more peptides as described herein;

(b) one or more polynucleotides encoding the peptide in an expressible form; and

(c) one or more antigen-presenting cells and/or exosomes, which antigen-presenting cells and exosomes present a complex formed between a HLA antigen and a peptide as described herein on its surface.

[0010] It is a further object of the present ~~invention~~disclosure to provide methods for the treatment and/or prophylaxis of (i.e., preventing) cancers (tumors), and/or prevention of post-operative recurrence thereof, as well as methods for inducing CTLs, methods for inducing an immune response against tumor-associated endothelia and also anti-tumor immunity, which methods include the step of administering the MELK polypeptides,

polynucleotides encoding MELK polypeptides, exosomes or the antigen-presenting cells presenting MELK polypeptides or the pharmaceutical agents of the ~~invention~~disclosure. In addition, the CTLs of the ~~invention~~disclosure also find use as vaccines against cancer. Examples of the cancer include, but are not limited to breast cancer, bladder cancer, cervical cancer, cholangiocellular carcinomas, CML, colorectal cancer, endometriosis, esophagus cancer, gastric cancer, liver cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma and SCC.

[0011] It is to be understood that both the foregoing summary of the ~~invention~~ and the following detailed description are of exemplified embodiments, and not restrictive of the ~~invention~~disclosure or other alternate embodiments of the ~~invention~~disclosure.

Brief Description of Drawings

[0012] 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.

[fig.1]Figure 1 includes a series of photographs, (a) - (i) depicting the results of IFN-gamma ELISPOT assay on CTLs that were induced with peptides derived from MELK. The CTLs in well number #5 stimulated with MELK-A24-9-326 (SEQ ID NO: 14) (a), #3 with MELK-A24-9-78 (SEQ ID NO: 21) (b), #4 with MELK-A24-10-637 (SEQ ID NO: 23) (c), #4 with MELK-A24-10-532 (SEQ ID NO: 27) (d), #5 with MELK-A02-9-138 (SEQ ID NO: 36) (e), #3 with MELK-A02-9-193 (SEQ ID NO: 46) (f), #1 with MELK-A02-9-171 (SEQ ID NO: 57) (g), #2 with MELK-A02-9-71 (SEQ ID NO: 60) (h) and #2 with MELK-A02-9-532 (SEQ ID NO: 62) (i) showed potent IFN-gamma production as compared with the control, respectively. The cells in the wells denoted with a rectangular box were expanded to establish CTL lines. In the figures, "+" indicates that the target cells in the well were pulsed with the appropriate peptide, and "-" indicates that the target cells had not been pulsed with any peptides.

[fig.2]Figure 2 includes a series of line graphs, (a) - (i), depicting the IFN-gamma production of CTL lines stimulated with SEQ ID NO: 14 (a), SEQ ID NO: 21 (b), SEQ ID NO: 23 (c), SEQ ID NO: 27 (d), SEQ ID NO: 36 (e), SEQ ID NO: 46 (f), SEQ ID NO: 57 (g), SEQ ID NO: 60 (h) and SEQ ID NO: 62 (i) with IFN-gamma ELISA assay. CTL lines established by stimulation with each peptide showed potent IFN-gamma production as compared with the control. In the figures, "+" indicates that the target cells were pulsed with the appropriate peptide and "-" indicates that the target cells had not been pulsed with any peptides.

[fig.3]Figure 3 depicts the IFN-gamma production of the CTL clone established by limiting dilution from the CTL line stimulated with SEQ ID NO: 27. The results

Claims

- [Claim 1] An isolated peptide derived from SEQ ID NO: 94, wherein said peptide comprises an amino acid sequence selected from the group consisting of:
- (a) SEQ ID NOs: 14, 21, 23, 27, 36, 46, 57, 60 and 62; and
 - (b) SEQ ID NOs: 14, 21, 23, 27, 36, 46, 57, 60 and 62, wherein 1, 2, or several amino acids are substituted, inserted, deleted and/or added, and has cytotoxic T lymphocyte (CTL) inducibility.
- [Claim 2] The peptide as set forth in claim 1, wherein the peptide consists of less than 15 amino acid residues.
- [Claim 3] The peptide as set forth in claim 2, wherein the peptide is a nonapeptide or a decapeptide.
- [Claim 4] The peptide as set forth in any one of claims 1 to 3, wherein the peptide, comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 21, 23 and 27, has 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 5] The peptide as set forth in any one of claims 1 to 3, wherein the peptide, comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 36, 46, 57, 60 and 62, has one or both of the following characteristics:
- (a) the second amino acid from the N-terminus is selected from the group of leucine or methionine, and
 - (b) the C-terminal amino acid is selected from the group of valine or leucine.
- [Claim 6] An isolated polynucleotide encoding a peptide as set forth in any one of claims 1 to 5.
- [Claim 7] A pharmaceutical agent for the treatment and/or prophylaxis of cancer, and/or the prevention of a postoperative recurrence thereof, wherein the agent comprises an active ingredient selected from the group consisting of:
- (a) one or more peptides as set forth in any one of claims 1 to 5;
 - (b) one or more polynucleotides encoding the peptide in an expressible form;

- (c) one or more antigen-presenting cells and/or exosomes, which antigen-presenting cells and exosomes present a complex formed between a HLA antigen and a peptide as set forth in any one of claims 1 to 5 on its surface;
 - (d) one or more CTLs induced against a peptide as set forth in any one of claims 1 to 6; and
 - (e) combinations thereof..
- [Claim 8] The pharmaceutical agent as set forth in claim 7, formulated for the administration to a subject whose HLA antigen is HLA-A24 or HLA-A02.
- [Claim 9] The pharmaceutical agent as set forth in claim 7, formulated for the treatment of a cancer selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, chronic myeloid leukemia (CML), colorectal cancer, endometriosis, 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.
- [Claim 10] The pharmaceutical agent of claim 7, wherein said agent is formulated as a vaccine.
- [Claim 11] A method for inducing an antigen-presenting cell with high CTL inducibility, wherein the method comprises the step of contacting an antigen-presenting cell with a peptide as set forth in any one of claims 1 to 5, or the step of introducing a polynucleotide encoding the peptide in an expressible form into an antigen-presenting cell.
- [Claim 12] A method for inducing CTL by using a peptide as set forth in any one of claims 1 to 5.
- [Claim 13] The method for inducing CTL, wherein said method comprises the step selected from the group consisting of:
- (a) contacting a CD8-positive T cell with an antigen-presenting cell and/or an exosome which present a complex formed between an HLA antigen and a peptide as set forth in any one of claims 1 to 5 on its surface; and
 - (b) introducing a polynucleotide encoding a polypeptide which is capable of forming a TCR subunit recognizing a complex formed between an HLA antigen and a peptide as set forth in any one of claims 1 to 5 into a CD8-positive T cell.
- [Claim 14] An isolated CTL which targets any of the peptides of claims 1 to 5.
- [Claim 15] An isolated CTL which is induced by a peptide as set forth in any one

- of claims 1 to 5.
- [Claim 16] The CTL as set forth in claims 14 or 15, wherein said CTL is capable of recognizing a complex formed between an HLA antigen and a peptide as set forth in any one of claims 1 to 5 on a cell.
- [Claim 17] An isolated antigen-presenting cell that presents a complex formed between a HLA antigen and a peptide as set forth in any one of claims 1 to 5 on its surface.
- [Claim 18] The antigen-presenting cell as set forth in claim 17, which is induced by the method as set forth in claim 11.
- [Claim 19] The antigen-presenting cell as set forth in claims 17 or 18, wherein the HLA antigen is HLA-A24 or HLA-A02.
- [Claim 20] An agent for inducing an immune response against a cancer in a subject, wherein said agent comprises an active ingredient selected from the group consisting of:
- (a) one or more peptides as set forth in any one of claims 1 to 5;
 - (b) one or more polynucleotides encoding the peptide in an expressible form;
 - (c) one or more antigen-presenting cells and/or exosomes, which antigen-presenting cells and exosomes present a complex formed between a HLA antigen and a peptide as set forth in any one of claims 1 to 5 on its surface;
 - (d) one or more CTLs induced against a peptide as set forth in any one of claims 1 to 6; and
 - (e) combinations thereof.
- [Claim 21] A method of inducing an immune response against a cancer in a subject, wherein said method comprising the step of administering to said subject an agent comprising an active ingredient selected from the group of consisting of:
- (a) one or more peptides as set forth in any one of claims 1 to 5;
 - (b) one or more polynucleotides encoding the peptide in an expressible form;
 - (c) one or more antigen-presenting cells and/or exosomes, which antigen-presenting cells and exosomes present a complex formed between a HLA antigen and a peptide as set forth in any one of claims 1 to 5 on its surface;
 - (d) one or more CTLs induced against a peptide as set forth in any one of claims 1 to 6; and
 - (e) combinations thereof.

[Claim 22]

The method as set forth in claim 21, wherein said cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, endometriosis, esophagus cancer, gastric cancer, liver cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma and small cell lung cancer.

[Claim 23]

The method as set forth in claim 21 or 22, wherein the subject has HLA-A24 or HLA-A02.

CLAIMS:

1. An isolated peptide of less than 15 amino acids, wherein said peptide comprises an amino acid sequence selected from the group consisting of:
 - (a) SEQ ID NOs:36, 46, 57, 60 and 62; and
 - (b) SEQ ID NOs:36, 46, 57, 60 and 62, in which 1 or 2 amino acids are substituted, inserted, deleted and/or added, and has cytotoxic T lymphocyte (CTL) inducibility.
2. The peptide as set forth in Claim 1, wherein the peptide is a nonapeptide or a decapeptide.
3. The peptide as set forth in Claim 1 or 2, 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: 36, 46, 57, 60 or 62 is substituted with an amino acid selected from the group of leucine and methionine; and
 - (b) the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 36, 46, 57, 60 or 62 is substituted with an amino acid selected from the group of valine and leucine.
4. The peptide as set forth in Claim 1 or 2, wherein said peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs:36, 46, 57, 60 and 62.
5. An isolated polynucleotide encoding a peptide as set forth in any one of Claims 1 to 4.
6. A pharmaceutical composition for the treatment and/or prophylaxis of cancer, and/or the prevention of a postoperative recurrence thereof, wherein the pharmaceutical composition comprises an active ingredient selected from the group consisting of:
 - (a) one or more peptides as set forth in any one of Claims 1 to 4;
 - (b) one or more polynucleotides encoding the peptide in an expressible form;

- (c) one or more antigen-presenting cells and/or exosomes, which antigen-presenting cells and exosomes present a complex formed between a HLA antigen and a peptide as set forth in any one of Claims 1 to 4 on its surface;
- (d) one or more CTLs induced against a peptide as set forth in any one of Claims 1 to 4; and
- (e) combinations thereof.

7. The pharmaceutical composition as set forth in Claim 6, wherein the pharmaceutical composition is formulated for the administration to a subject whose HLA antigen is HLA-A02.

8. The pharmaceutical composition as set forth in Claim 6, wherein the pharmaceutical composition is formulated for the treatment of a cancer selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, chronic myeloid leukemia (CML), colorectal cancer, endometriosis, 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.

9. The pharmaceutical composition of Claim 6, wherein said pharmaceutical composition is formulated as a vaccine.

10. An *in vitro* method for inducing an antigen-presenting cell with high CTL inducibility, wherein the method comprises the step of contacting an antigen-presenting cell with a peptide as set forth in any one of Claims 1 to 4, or the step of introducing a polynucleotide encoding the peptide in an expressible form into an antigen-presenting cell.

11. An *in vitro* method for inducing CTL by using a peptide as set forth in any one of Claims 1 to 4.

12. The method for inducing CTL as set forth in Claim 11, wherein said method comprises the step selected from the group consisting of:

(a) contacting a CD8-positive T cell with an antigen-presenting cell and/or an exosome which present a complex formed between an HLA antigen and a peptide as set forth in any one of Claims 1 to 4 on its surface; and

(b) introducing a polynucleotide encoding a polypeptide which is capable of forming a TCR subunit recognizing a complex formed between an HLA antigen and a peptide as set forth in any one of Claims 1 to 4 into a CD8-positive T cell.

13. An isolated CTL which targets any of the peptides of Claims 1 to 4.

14. An isolated CTL which is induced by a peptide as set forth in any one of Claims 1 to 4.

15. The CTL as set forth in Claims 13 or 14, wherein said CTL is capable of recognizing a complex formed between an HLA antigen and a peptide as set forth in any one of Claims 1 to 4 on a cell.

16. The CTL as set forth in any one of Claims 13 to 15, which is induced by the method as set forth in Claim 12.

17. An isolated antigen-presenting cell that presents a complex formed between a HLA antigen and a peptide as set forth in any one of Claims 1 to 4 on its surface.

18. The antigen-presenting cell as set forth in Claim 17, which is induced by the method as set forth in Claim 10.

19. The antigen-presenting cell as set forth in Claim 17 or 18, wherein the HLA antigen is HLA-A02.

20. A composition for inducing an immune response against a cancer in a subject, wherein said composition comprises an active ingredient selected from the group consisting of:

- (a) one or more peptides as set forth in any one of Claims 1 to 4;
- (b) one or more polynucleotides encoding the peptide in an expressible form;
- (c) one or more antigen-presenting cells and/or exosomes, which antigen-presenting cells and exosomes present a complex formed between a HLA antigen and a peptide as set forth in any one of Claims 1 to 4 on its surface;
- (d) one or more CTLs induced against a peptide as set forth in any one of Claims 1 to 4; and
- (e) combinations thereof.

21. A composition for inducing a CTL, wherein said composition comprises an active ingredient selected from the group consisting of:

- (a) one or more peptides as set forth in any one of Claims 1 to 4;
 - (b) one or more polynucleotides encoding the peptide in an expressible form;
- and
- (c) one or more antigen-presenting cells and/or exosomes, which antigen-presenting cells and exosomes present a complex formed between a HLA antigen and a peptide as set forth in any one of Claims 1 to 4 on its surface.