



US 20210371462A1

(19) **United States**(12) **Patent Application Publication**
YAMASHITA et al.(10) **Pub. No.: US 2021/0371462 A1**(43) **Pub. Date: Dec. 2, 2021**(54) **CDCA1-DERIVED PEPTIDE AND VACCINE
CONTAINING SAME***A61K 35/15* (2006.01)*C07K 14/74* (2006.01)*A61P 35/00* (2006.01)(71) Applicant: **ONCOTHERAPY SCIENCE, INC.**,
Kawasaki-shi, Kanagawa (JP)(52) **U.S. Cl.**CPC *C07K 7/06* (2013.01); *A61K 35/17*
(2013.01); *A61K 38/00* (2013.01); *C07K*
14/70539 (2013.01); *A61P 35/00* (2018.01);
A61K 35/15 (2013.01)(72) Inventors: **SACHIKO YAMASHITA**,
Kawasaki-shi (JP); **Tetsuro HIKICHI**,
Kawasaki-shi, Kanagawa (JP)(73) Assignee: **ONCOTHERAPY SCIENCE, INC.**,
Kawasaki-shi, Kanagawa (JP)

(57)

ABSTRACT(21) Appl. No.: **17/264,277**(22) PCT Filed: **Aug. 1, 2019**(86) PCT No.: **PCT/JP2019/030117**

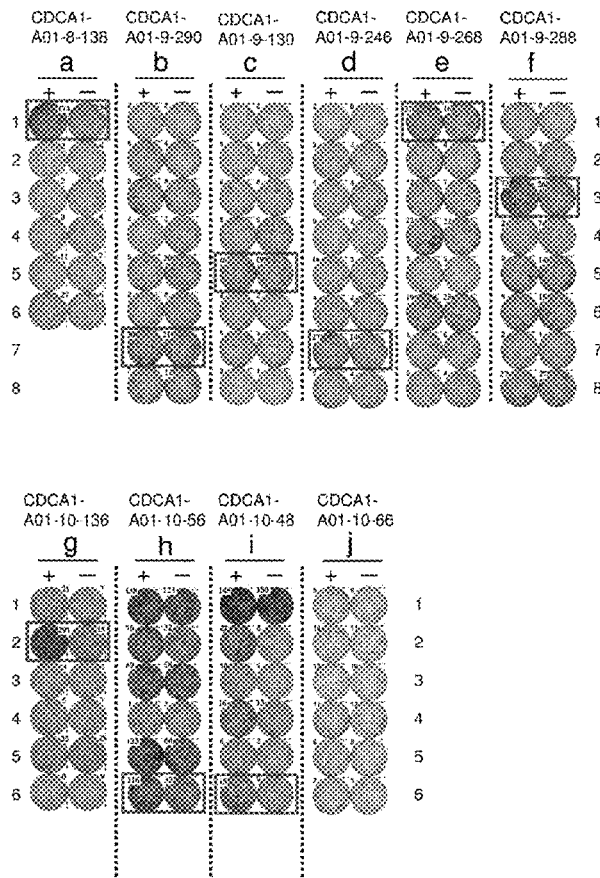
§ 371 (c)(1),

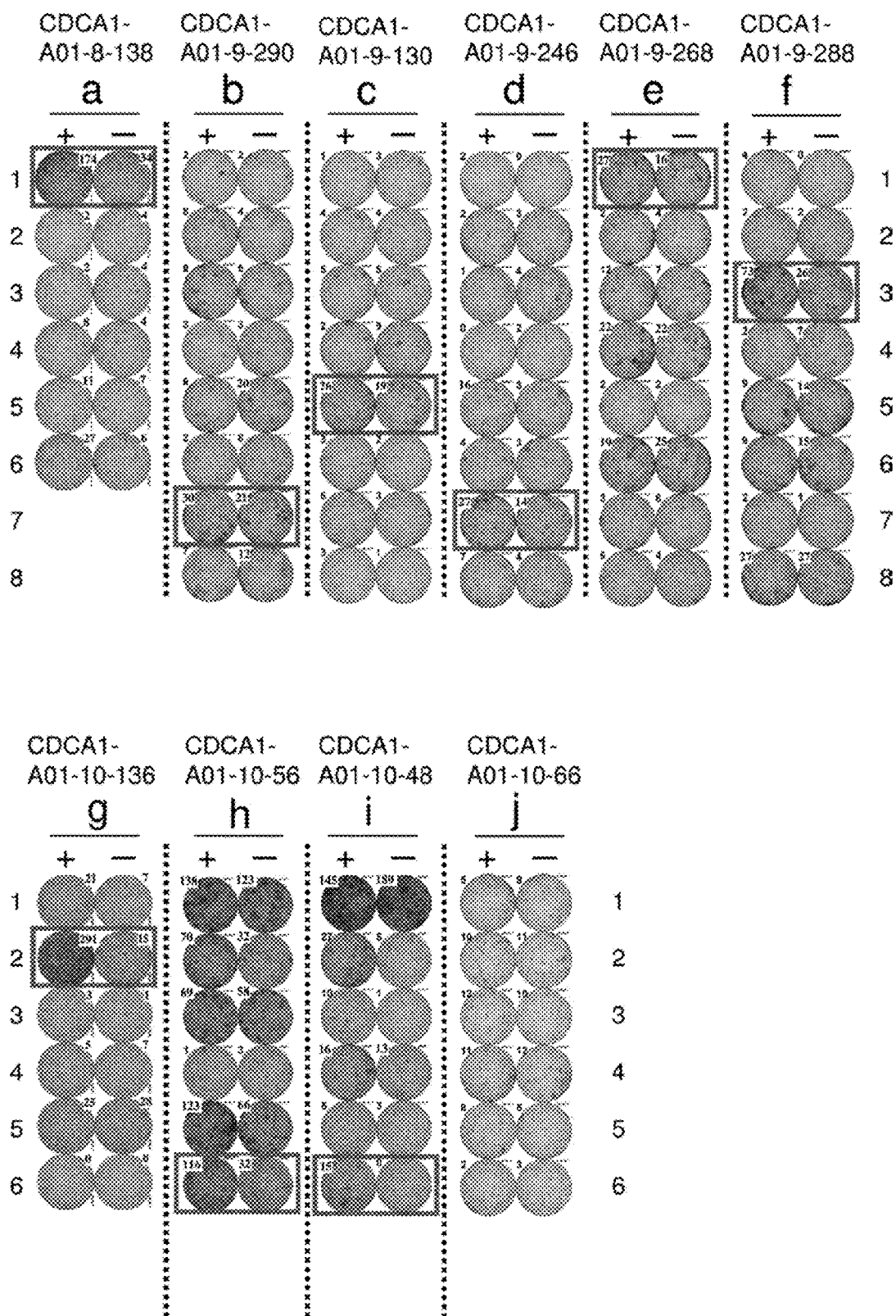
(2) Date: **Jan. 28, 2021**(30) **Foreign Application Priority Data**

Aug. 2, 2018 (JP) 2018-145607

Publication Classification(51) **Int. Cl.***C07K 7/06* (2006.01)*A61K 35/17* (2006.01)

The present invention provides CDCA1-derived epitope peptides having the ability to induce cytotoxic T cells. The present invention further provides polynucleotides encoding the peptides, antigen-presenting cells presenting the peptides, and cytotoxic T cells targeting the peptides, as well as methods of inducing the antigen-presenting cells or CTLs. The present invention also provides compositions and pharmaceutical compositions containing them as an active ingredient. Further, the present invention provides methods of treating and/or preventing cancer, and/or preventing post-operative recurrence thereof, using the peptides, polynucleotides, antigen-presenting cells, cytotoxic T cells or pharmaceutical compositions of the present invention. Methods of inducing an immune response against cancer are also provided.

Specification includes a Sequence Listing.



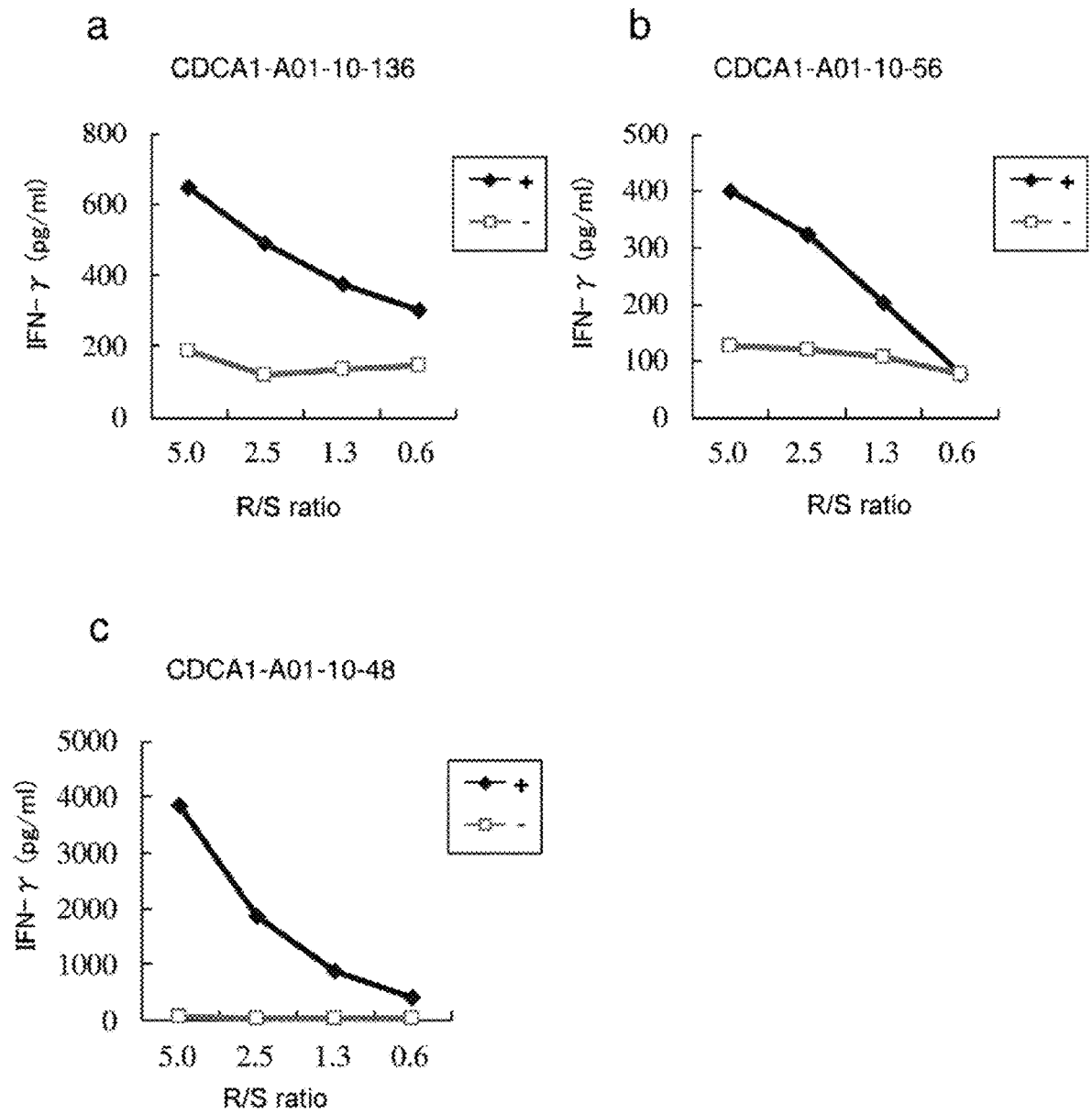


FIG. 2

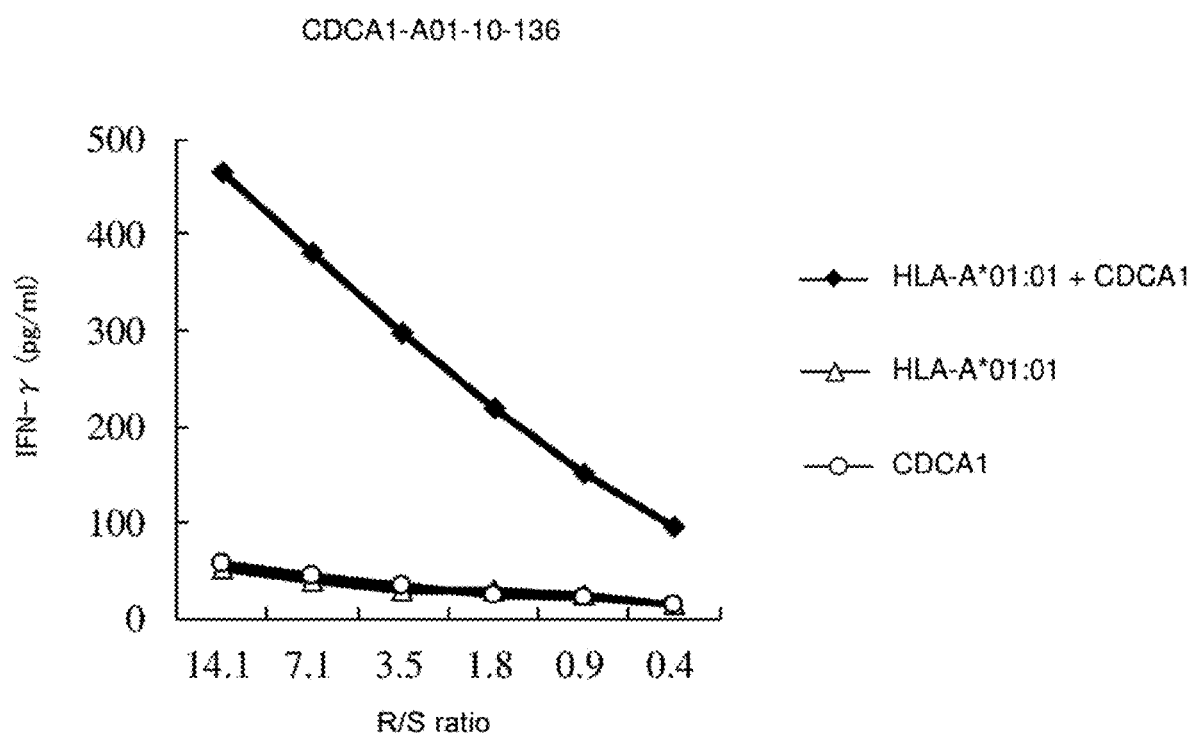


FIG. 3

CDCA1-DERIVED PEPTIDE AND VACCINE CONTAINING SAME

TECHNICAL FIELD

[0001] The present invention relates to the field of biological science, more specifically to the field of cancer therapy. In particular, the present invention relates to novel peptides that are effective as cancer vaccines, methods for either or both of treating and preventing cancers using the peptide(s), and pharmaceutical compositions comprising the peptide(s).

BACKGROUND ART

[0002] Cytotoxic T lymphocytes (CTLs) have been known to recognize epitope peptides derived from the tumor-associated antigens (TAAs) presented on the major histocompatibility complex (MHC) class I molecule, which is expressed on tumor cell surface, and then kill the tumor cells. To date, many TAAs including the melanoma antigen (MAGE) family have been discovered through immunological approaches (NPL1: Boon T, *Int J Cancer* 1993, 54(2): 177-80; NPL2: Boon T & van der Bruggen P, *J Exp Med* 1996, 183(3):725-9). Immunotherapies targeting these TAAs are currently undergoing clinical development.

[0003] In some TAAs, epitope peptides that can be recognized by CTLs were identified and their application on immunotherapy for various cancers is anticipated (NPL3: Harris C C, *J Natl Cancer Inst* 1996, 88(20): 1442-55; NPL4: Butterfield L H et al., *Cancer Res* 1999, 59(13): 3134-42; NPL5: Vissers J L et al., *Cancer Res* 1999, 59(21): 5554-9; NPL6: van der Burg S H et al., *J Immunol* 1996, 156(9): 3308-14; NPL7: Tanaka F et al., *Cancer Res* 1997, 57(20): 4465-8; NPL8: Fujie T et al., *Int J Cancer* 1999, 80(2): 169-72; NPL9: Kikuchi M et al., *Int J Cancer* 1999, 81(3): 459-66; NPL10: Oiso M et al., *Int J Cancer* 1999, 81(3): 387-94). Until now, several clinical trials for cancer immunotherapy using these TAA-derived CTL epitope peptides have been reported. Unfortunately, however, in many of these clinical trials, the response rate is not necessarily high (NPL11: Belli F et al., *J Clin Oncol* 2002, 20(20): 4169-80; NPL12: Coulie P G et al., *Immunol Rev* 2002, 188: 33-42; NPL13: Rosenberg SA et al., *Nat Med* 2004, 10(9): 909-15). Therefore, there is still demand for identification of novel epitope peptides that are applicable to cancer immunotherapy.

[0004] CDCA1 (cell division cycle associated 1; also described as NUF2, NDC80 kinetochore complex component: Nuf2; reference sequence: GeneBank Accession Number NM_145697 (SEQ ID NO: 44) or GeneBank Accession Number NM_031423 (SEQ ID NO: 46)) has been identified as a member of the genes co-expressed with CDC2, cyclin, topoisomerase II and other cell cycle genes (NPL14: Walker et al., *Curr Cancer Drug Targets* 2001, 1(1): 73-83). CDCA1 has been found to be related to the centromere of HeLa cells undergoing mitotic division, and is considered to be a functional homologue of yeast Nuf2 (NPL15: Wigge P A et al., *J Cell Biol* 2001, 152(2): 349-60). Meanwhile, CDCA1 has been identified as a gene showing elevated expression in non-small cell lung cancer by gene expression profiling based on a genome-wide cDNA microarray targeting 27648 genes (NPL16: Hayama et al., *Cancer Res* 2006, 66(21): 10339-48; PTL1: WO2007/013480; PTL2: WO2005/089735). The CDCA1 expression is observed in lung cancer

tissues and lung cancer cell lines, but little expression is observed in 22 normal tissues except the testis (NPL16; PTL1). Further, as a result of siRNA-mediated suppression of the CDCA1 expression, suppression of cell proliferation in CDCA1-expressing lung cancer cell lines is caused (NPL16; PTLs 1-2). Moreover, elevated expression of CDCA1 is also observed in various cancers such as cholangiocellular cancer, bladder cancer and renal cell cancer (NPL17: Harao M et al., *Int J Cancer* 2008, 123(11): 2616-25).

[0005] Recently, CDCA1-derived HLA-A02-restricted CTL epitope peptides (NPL17: Harao et al., *Int J Cancer* 2008, 123(11): 2616-25; PTL3: WO2009/025117), HLA-A24-restricted CTL epitope peptides (PTL4: WO2009/153992), HLA-A11-restricted CTL epitope peptides (PTL5: WO2016/021508), HLA-A33-restricted CTL epitope peptides (PTL6: WO2016/021508) and HLA-A03-restricted CTL epitope peptides (PTL7: WO2016/021508) have been identified. Therapeutic effects by these peptides are expected in cancer patients having the HLA-A02 type, HLA-A24 type, HLA-A11 type, HLA-A33 type or HLA-A03 type, but for cancer patients having HLA types other than those, peptides corresponding to respective HLA type are desired.

CITATION LIST

Patent Literature

- [0006]** [PTL 1] WO2007/013480
- [0007]** [PTL 2] WO2005/089735
- [0008]** [PTL 3] WO2009/025117
- [0009]** [PTL 4] WO2009/153992
- [0010]** [PTL 5] WO2016/021508

Non Patent Literature

- [0011]** [NPL 1] Boon T, *Int J Cancer* 1993, 54(2): 177-80
- [0012]** [NPL 2] Boon T & van der Bruggen P, *J Exp Med* 1996, 183(3): 725-9
- [0013]** [NPL 3] Harris CC, *J Natl Cancer Inst* 1996, 88(20): 1442-55
- [0014]** [NPL 4] Butterfield L H et al., *Cancer Res* 1999, 59(13): 3134-42
- [0015]** [NPL 5] Vissers J L et al., *Cancer Res* 1999, 59(21): 5554-9
- [0016]** [NPL 6] van der Burg S H et al., *J Immunol* 1996, 156(9): 3308-14
- [0017]** [NPL 7] Tanaka F et al., *Cancer Res* 1997, 57(20): 4465-8
- [0018]** [NPL 8] Fujie T et al., *Int J Cancer* 1999, 80(2): 169-72
- [0019]** [NPL 9] Kikuchi M et al., *Int J Cancer* 1999, 81(3): 459-66
- [0020]** [NPL 10] Oiso M et al., *Int J Cancer* 1999, 81(3): 387-94
- [0021]** [NPL 11] Belli F et al., *J Clin Oncol* 2002, 20(20): 4169-80
- [0022]** [NPL 12] Coulie PG et al., *Immunol Rev* 2002, 188: 33-42
- [0023]** [NPL 13] Rosenberg SA et al., *Nat Med* 2004, 10(9): 909-15
- [0024]** [NPL 14] Walker et al., *Curr Cancer Drug Targets* 2001, 1(1): 73-83
- [0025]** [NPL 15] Wigge PA et al., *J Cell Biol* 2001, 152(2): 349-60

[0026] [NPL 16] Hayama et al., *Cancer Res* 2006, 66(21): 10339-48

[0027] [NPL 17] Harao et al., *Int J Cancer* 2008, 123(11): 2616-25

SUMMARY OF THE INVENTION

[0028] The present invention relates to peptides that can induce cytotoxic T cells (CTLs) specifically responding to CDCA1-expressing cells. When these peptides are presented on antigen-presenting cells (APCs) by the human leukocyte antigen (HLA) and recognized by CD8-positive T cells, CTLs that show a peptide-specific cytotoxic activities are induced. CDCA1-derived peptides that have been identified so far to have CTL-inducing ability (CTL inducibility) are HLA-A02-restricted, HLA-A24-restricted, HLA-A11-restricted, HLA-A33-restricted, or HLA-A03-restricted peptides, and when antigen-presenting cells do not express these HLAs, the peptides cannot induce CTLs. Therefore, conventional peptides are not suitable for performing immunotherapy on cancer patients (subjects) that do not have these HLAs. HLA-A01 is an HLA allele highly frequently observed in Caucasians (Cao K et al., *Hum Immunol* 2001, 62(9): 1009-30). It is desirable to administer HLA-A01-restricted peptides to HLA-A01-positive cancer patients. Hence, the present invention relates to CDCA1-derived peptides with CTL-inducing ability that are restrictive to HLA-A01. Based on results disclosed herein, the peptides of the present invention have been proven to be epitope peptides that can induce a potent and specific immune response against cancer cells expressing CDCA1 and HLA A01.

[0029] Therefore, one of the objectives of the present invention is to provide CDCA1-derived peptides that can induce CTLs in an HLA-A01-restrictive manner. These peptides can be used to induce CTLs in vitro, ex vivo or in vivo, or can be used to administer to subjects for the purpose of inducing an immune response against CDCA1-expressing cancer cells. Preferable peptides are peptides comprising the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37; more preferable peptides are nonapeptides or decapeptides; and even more preferable peptides are peptides consisting of the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37.

[0030] The peptides of the present invention encompass peptides in which one, two, or more amino acid(s) is/are substituted, deleted, inserted and/or added, as long as the resultant modified peptides retain the CTL-inducing ability of the original peptide.

[0031] The present invention further provides isolated polynucleotides encoding any one of the peptides of the present invention. Similar to the peptides of the present invention, these polynucleotides can be used for inducing APCs with CTL-inducing ability, and can be administered to subjects for inducing an immune response against CDCA1-expressing cancer cells.

[0032] The present invention also provides compositions comprising one or more types of peptides of the present invention, one or more types of polynucleotides encoding one or more types of peptides of the present invention, APCs of the present invention, exosomes presenting peptides of the present invention, and/or CTLs of the present invention. The compositions of the present invention are preferably pharmaceutical compositions. The pharmaceutical compositions of the present invention can be used for treating

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

[0033] A further objective of the present invention is to provide methods of inducing APCs having CTL-inducing ability, wherein the methods comprise a step of contacting one or more types of peptides of the present invention with an APC, or a step of introducing a polynucleotide encoding any one peptide of the present invention into an APC.

[0034] The present invention further provides a method of inducing CTLs, comprising a step of co-culturing a CD8-positive T cell with an APC that presents on its surface a complex of an HLA antigen and a peptide of the present invention, a step of co-culturing a CDR-positive T cell with an exosome that presents on its surface a complex of an HLA antigen and a peptide of the present invention, or a step of introducing into a CD8-positive T cell a vector comprising a polynucleotide encoding each subunit of a T cell receptor (TCR) capable of binding to a peptide of the present invention presented by an HLA antigen on a cell surface.

[0035] A further objective of the present invention is to provide isolated APCs that present on their surface a complex of an HLA antigen and a peptide of the present invention. The present invention further provides isolated CTLs targeting a peptide of the present invention. These APCs and CTLs can be used in immunotherapy for CDCA1-expressing cancers.

[0036] Another objective of the present invention is to provide methods of inducing an immune response against cancer in a subject, wherein the methods comprise a step of administering to the subject a composition(s) comprising a peptide(s) of the present invention or a polynucleotide(s) encoding the peptide(s), an APC(s) of the present invention, an exosome(s) presenting a peptide(s) of the present invention, and/or a CTL(s) of the present invention. Another objective of the present invention is to provide methods of treating and/or preventing cancer, as well as preventing postoperative recurrence thereof in a subject, wherein the methods comprise a step of administering to the subject a peptide(s) of the present invention, a polynucleotide(s) encoding the peptide(s), an APC(s) of the present invention, an exosome(s) presenting a peptide(s) of the present invention, and/or a CTL(s) of the present invention.

[0037] In addition to the above, other objects and features of the present invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying figures and examples. However, it is to be understood that both the foregoing summary of the present invention and the following detailed description are of exemplified embodiments, and not restrictive of the present invention or other alternate embodiments of the present invention. In particular, while the present invention is described herein with reference to a number of specific embodiments, it will be appreciated that the description is illustrative of the present invention and is not constructed as

limiting of the present invention. Various modifications and applications may occur to those who are skilled in the art, without departing from the spirit and the scope of the present invention, as described by the appended claims. Likewise, other objects, features, benefits and advantages of the present invention 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 THE DRAWINGS

[0038] FIG. 1 is comprised of photos (a) to (j) showing the results of the IFN-gamma enzyme-linked immunospot (ELISPOT) assay performed using cells induced using peptides derived from CDCA1. In the figure, “+” shows IFN-gamma production against target cells pulsed with a peptide of interest and “-” shows IFN-gamma production against target cells that have not been pulsed with any peptides (negative target). In comparison with the negative control, peptide-specific IFN-gamma production was observed in:

[0039] Well #1 with CDCA1-A01-8-138 (SEQ ID NO: 1) (a),

[0040] Well #7 with CDCA1-A01-9-290 (SEQ ID NO: 25) (b),

[0041] Well #5 with CDCA1-A01-9-130 (SEQ ID NO: 33) (c),

[0042] Well #7 with CDCA1-A01-9-246 (SEQ ID NO: 34) (d),

[0043] Well #1 with CDCA1-A01-9-268 (SEQ ID NO: 35) (e),

[0044] Well #3 with CDCA1-A01-9-288 (SEQ ID NO: 37) (f),

[0045] Well #2 with CDCA1-A01-10-136 (SEQ ID NO: 8) (g),

[0046] Well #6 with CDCA1-A01-10-56 (SEQ ID NO: 10) (h), and

[0047] Well #6 with CDCA1-A01-10-48 (SEQ ID NO: 13) (i).

[0048] CTL clones were established from the boxed cells in the photos that showed a reaction. In contrast, CDCA1-A01-10-66 (SEQ ID NO: 9) (j) is shown as an example of typical negative data where peptide-specific IFN-gamma production was not observed.

[0049] FIG. 2 is comprised of a series of line graphs (a) to (c) showing IFN-gamma production of CTL clones established by limiting dilution method following induction by CDCA1-A01-10-136 (SEQ ID NO: 8), CDCA1-A01-10-56 (SEQ ID NO: 10), or CDCA1-A01-10-48 (SEQ ID NO: 13). These results show peptide-specific IFN-gamma production of the CTL clones. In the figure, “+” shows IFN-gamma production of the CTL clones against target cells pulsed with a peptide of interest and “-” shows IFN-gamma production of the CTL clones against target cells that have not been pulsed with any peptides. The R/S ratio indicates the ratio of the cell number of the CTL clones (Responder cells) and the cell number of target cells that stimulate these cells (Stimulator cells).

[0050] FIG. 3 is a line graph showing IFN-gamma production of a CTL clone against target cells expressing both CDCA1 and HLA-A*01:01. COS7 cells expressing either HLA-A*01:01 or the full-length CDCA1 gene served as a

negative control. The CTL clone established following induction by CDCA1-A01-10-136 (SEQ ID NO: 8) showed IFN-gamma production in COS7 cells into which both CDCA1 and HLA-A*01:01 genes were introduced (black diamond). Meanwhile, it did not show significant IFN-gamma production in COS7 cells into which either HLA-A*01:01 (triangle) or CDCA1 (white circle) was introduced.

MODE FOR CARRYING OUT THE INVENTION

[0051] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. However, before the present materials and methods are described, it is to be understood that the present invention is not limited to the particular sizes, shapes, dimensions, materials, methodologies, protocols, etc. described herein, as these may vary in accordance with routine experimentation and optimization. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

I. Definitions

[0052] The words “a”, “an”, and “the” as used herein mean “at least one” unless otherwise specifically indicated.

[0053] The terms “isolated” and “purified” used in relation with a substance (for example, peptide, antibody, polynucleotide or such) indicate that the substance does not substantially contain at least one substance that may else be included in a natural source. Thus, an isolated or purified peptide refers to a peptide that does not substantially contain another cellular material, for example, carbohydrate, lipid and other contaminating proteins from the cell or tissue source from which the peptide is derived. When the peptide is chemically synthesized, an isolated or purified peptide refers to a peptide that does not substantially contain a precursor substance or another chemical substance. The phrase “does not substantially contain a cellular material” includes peptide preparations in which the peptide is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a peptide that does not substantially contain a cellular material encompasses peptide preparations that contain less than about 30%, 20%, 10%, or 5%, 3%, 2% or 1% (dry weight basis) of other cellular materials. When the peptide is recombinantly produced, an isolated or purified peptide does not substantially contain culture medium, which encompasses peptide preparations that contain culture medium less than about 20%, 10%, or 5%, 3%, 2% or 1% (dry weight basis) of the volume of the peptide preparation. When the peptide is generated by chemical synthesis, an isolated or purified peptide does not substantially contain a precursor substance or other chemical substances, which encompasses peptide preparations that contain a precursor substance or other chemical substances less than about 30%, 20%, 10%, 5%, 3%, 2% or 1% (dry weight basis) of the volume of the peptide preparation. That a particular peptide preparation is an isolated or purified peptide can be confirmed, for example, by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electro-

phoresis and Coomassie Brilliant Blue staining or such of the gel. In a preferred embodiment, the peptides and polynucleotides of the present invention are isolated or purified.

[0054] The terms “polypeptide”, “peptide” and “protein” are used interchangeably herein, and refer to polymers of amino acid residues. These terms are applied to also non-naturally occurring amino acid polymers comprising one or more non-naturally occurring amino acid residues, in addition to naturally occurring amino acid polymers. Non-naturally occurring amino acids include amino acid analogs, amino acid mimetics, and such.

[0055] The term “amino acid” as used herein refers to naturally occurring amino acids, as well as amino acid analogs and amino acid mimetics that functions similarly to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those modified after translation in cells (e.g., hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine, etc.). The phrase “amino acid analog” refers to compounds that have the same basic chemical structure (an alpha carbon bound to a hydrogen, a carboxy group, an amino group, and an R group) as a naturally occurring amino acid but have a modified R group or modified backbones (e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium, and such). The phrase “amino acid mimetic” refers to chemical compounds that have different structures but similar functions to general amino acids. Amino acids can be either L-amino acids or D-amino acids, and the peptides of the present invention are preferably L-amino acid polymers.

[0056] The terms “polynucleotide”, “oligonucleotide” and “nucleic acid” are used interchangeably herein, and refer to a polymer of nucleotides.

[0057] The term “composition” used in the present specification is intended to encompass products that include specified ingredients in specified amounts, and any products generated directly or indirectly from combination of specified ingredients in the specified amounts. When the composition is a pharmaceutical composition, the term “composition” is intended to encompass products including active ingredient(s) and inert ingredient(s), as well as any products generated directly or indirectly from combination, complexation or aggregation of any two or more ingredients, from dissociation of one or more ingredients, or from other types of reactions or interactions of one or more ingredients. Thus, the pharmaceutical compositions of the present invention encompass any compositions made by admixing compounds or cells of the present invention with a pharmaceutically or physiologically acceptable carrier. Without being limited thereto, the terms “pharmaceutically acceptable carrier” or “physiologically acceptable carrier” used in the present specification include liquid or solid bulking agents, diluents, excipients, solvents, and encapsulation materials; and mean pharmaceutically or physiologically acceptable materials, compositions, substances or media.

[0058] Unless otherwise specified, the term “cancer” refers to a cancer that overexpresses the CDCA1 gene; and examples thereof include bladder cancer, breast cancer, cervical cancer, cholangiocellular cancer, chronic myeloid leukemia (CML), esophagus cancer, gastric cancer, non-small-cell lung cancer, lymphoma, osteosarcoma, prostate cancer, kidney cancer, small-cell lung cancer, head and neck cancer, soft tissue tumor, colon cancer and such, without being limited thereto. In an exemplary embodiment, the “cancer” is a cancer that expresses CDCA1 and HLA-A01.

[0059] Unless otherwise specified, the terms “cytotoxic T lymphocyte” and “cytotoxic T cell” and “CTL” are used interchangeably herein. Unless otherwise specifically indicated, they refer to a sub-group of T lymphocytes that can recognize non-self cells (for example, tumor/cancer cells, virus-infected cells) and induce the death of such cells.

[0060] Unless otherwise specified, the term “HLA-A01 (HLA-A1)” refers to the HLA-A01 type which includes subtypes such as HLA-A*01:01, HLA-A*01:02, HLA-A*01:03, and HLA-A*01:04.

[0061] In the context of a subject or patient, the phrase “HLA antigen of a subject (or patient) is HLA-A01” used herein refers to that a subject or patient has the HLA-A01 antigen gene homozygously or heterozygously as the MHC (Major Histocompatibility Complex) Class I molecule, and that the HLA-A01 antigen is expressed in the cells of the subject or patient as the HLA antigen.

[0062] As long as the methods and compositions of the present invention are useful in the context of cancer “treatment”, the treatment is considered “efficacious” when it achieves clinical advantages, for example, reduction in the size, spreading or metastatic ability of cancer, retardation of cancer progression, alleviation of clinical symptoms of cancer, prolongation of survival period, suppression of post-operative recurrence in a subject. When the treatment is applied prophylactically, “efficacious” means that the treatment retards or prevents cancer formation, or prevents or alleviates clinical symptoms of cancer. Effectiveness is determined in relation to any publicly known method for diagnosing or treating a specific tumor type.

[0063] As long as the methods and compositions of the present invention are useful in the context of cancer “prevention (prophylaxis)”, the term “prevention (prophylaxis)” herein includes any work that eases the load of disease-associated mortality or morbidity. Prevention (Prophylaxis) can be carried out at the “primary, secondary and tertiary prevention (prophylaxis) levels”. Whereas the primary prevention (prophylaxis) avoids the development of a disease, prevention (prophylaxis) at the secondary and tertiary levels encompasses prevention (prophylaxis) of disease progression and appearance of symptoms, as well as workings intended to reduce adverse effects of the existing disease by restoring functions and reducing disease-associated complications. Alternately, prevention (prophylaxis) can include alleviation of severity of a specific disorder, for example, extensive preventive therapy intended to reduce tumor growth and metastasis.

[0064] In the context of the present invention, the treatment and/or prevention (prophylaxis) of cancer and/or prevention (prophylaxis) of postoperative recurrence thereof include either of the events such as inhibition of cancer cell proliferation, tumor involution or regression, induction of remission and suppression of cancer development, tumor regression, as well as reduction or inhibition of metastasis, suppression of postoperative recurrence of cancer, and prolongation of survival period. Effective treatment and/or prevention (prophylaxis) of cancer reduce mortality, improve prognosis of an individual with cancer, reduce the blood levels of tumor markers, and alleviate detectable symptoms associated with cancer. For example, alleviation or improvement of symptoms constitutes effective treatment and/or prevention (prophylaxis), and includes a condition in which the symptoms are alleviated or stable by 10%, 20%, 30% or more.

[0065] In the context of the present invention, the term “antibody” refers to immunoglobulins and fragments thereof that are specifically reactive to a designated protein or peptide thereof. An antibody can include human antibodies, primatized antibodies, chimeric antibodies, bispecific antibodies, humanized antibodies, antibodies fused to other proteins or radiolabels, and antibody fragments. Furthermore, an “antibody” herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies) formed from two or more intact antibodies, and antibody fragments so long as they exhibit the desired biological activity. An “antibody” may be antibodies of all classes (e.g., IgA, IgD, IgE, IgG and IgM).

[0066] Unless otherwise specified, the technical terms and scientific terms used herein all have the same meanings as terms commonly understood by one of ordinary skill in the art to which the present invention belongs.

II. Peptides

[0067] HLA-A01 is an HLA allele commonly seen in Caucasians (Cao et al., Hum Immunol 2001, 62(9): 1009-30). Thus, an effective method of treating CDCA1-expressing cancers for a great population of Caucasians can be provided by providing CDCA1-derived CTL-inducing peptides restricted to HLA-A01. Thus, the present invention provides CDCA1-derived peptides that are capable of inducing CTLs in an HLA-A01-restrictive manner.

[0068] The peptides of the present invention are CDCA1-derived peptides that are capable of inducing CTLs in an HLA-A01-restrictive manner. Peptides capable of inducing CTLs in an HLA-A01-restrictive manner include peptides having the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37.

[0069] CTLs having a cytotoxic activity specific to these peptides can be established by in vitro stimulation of T cells by dendritic cells pulsed with these peptides. The established CTLs show a specific cytotoxic activity against target cells pulsed with each of the peptides.

[0070] The CDCA1 gene is overexpressed in cancer cells such as cancer cells in, for example, bladder cancer, breast cancer, cervical cancer, cholangiocellular cancer, chronic myeloid leukemia (CML), esophagus cancer, gastric cancer, non-small-cell lung cancer, lymphoma, osteosarcoma, prostate cancer, kidney cancer, small-cell lung cancer, head and neck cancer, soft tissue tumor, colon cancer and such, but is not expressed in most normal organs. It is thus an excellent target for immunotherapy. Therefore, the peptides of the present invention can be suitably used for cancer immunotherapy. A preferred peptide is a nonapeptide (a peptide consisting of 9 amino acid residues) or a decapeptide (a peptide consisting of 10 amino acid residues), and it is more preferably a peptide consisting of the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37. For example, a peptide having the amino acid sequence of SEQ ID NO: 8 is suitable for induction of CTLs that show a specific cytotoxic activity against cells expressing HLA-A01 and CDCA1, and can be suitably used for cancer immunotherapy in HLA-A01-positive patients. In a more preferred embodiment, the peptide of the present invention is a peptide consisting of the amino acid sequence of SEQ ID NO: 8.

[0071] For the peptides of the present invention, an additional amino acid residue(s) can be made to adjoin the amino

acid sequence of the peptide of the present invention, as long as the resultant peptides retain the CTL-inducing ability of the original peptide. The additional amino acid residue(s) may be composed of any types of amino acid(s), as long as they do not impair the CTL-inducing ability of the original peptide. Therefore, the peptides of the present invention encompass peptides having CTL-inducing ability, comprising the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37. Such peptides are, for example, less than about 40 amino acids, in many cases less than about 20 amino acids, and usually less than about 15 amino acids. Therefore, if the original peptide is a nonapeptide, the peptide of the present invention encompasses peptides that are 10 amino-acid long or 11-40 amino-acid long, which are produced by adjoining additional amino acid(s) to the peptide. Furthermore, if the original peptide is a decapeptide, the peptide of the present invention encompasses peptides that are 11-40 amino-acid long. Such a peptide can be, for example, a peptide that is 11-20 amino-acid long or a peptide that is 11-15 amino-acid long. A preferred example of an additional amino acid residue(s) is an amino acid residue(s) adjacent to the amino acid sequence of the peptide of the present invention in the full-length amino acid sequence of CDCA1 (for example, SEQ ID NO: 64). Therefore, the peptides of the present invention encompass peptides comprising the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37, and wherein the peptides are peptide fragments of CDCA1 and have CTL-inducing ability.

[0072] In general, modifications of one, two or more amino acids in a certain peptide do not affect the functions of the peptide, or in some cases even enhance the desired functions of the original peptide. In fact, modified peptides (i.e., peptides composed of the amino acid sequence in which one, two or several amino acid residues are modified (i.e., substituted, deleted, inserted, and/or added) compared to the original reference sequence) are known to retain the biological activity of the original peptide (Mark et al., Proc Natl Acad Sci USA 1984, 81: 5662-6; Zoller and Smith, Nucleic Acids Res 1982, 10: 6487-500; Dalbadie-McFarland et al., Proc Natl Acad Sci USA 1982, 79: 6409-13). Thus, in one embodiment, the peptides of the present invention can be peptides comprising the amino acid sequence in which one, two or several amino acids are substituted, deleted, inserted and/or added to the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37 and having CTL-inducing ability.

[0073] On skilled in the art can recognize that individual substitutions to an amino acid sequence that alter a single amino acid or a small percentage of amino acids tend to result in the conservation of the properties of the original amino acid side chain(s). Accordingly, those are frequently referred to as “conservative substitutions” or “conservative modifications”; and modification of a protein by “conservative substitution” or “conservative modification” may result in a modified protein that has similar functions as the original protein. Tables of conservative substitutions presenting functionally similar amino acids are well known in the art. Examples of amino acid side chain characteristics that functionally resemble include, for example, hydrophobic amino acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), and side chains having the following functional groups or characteristics in common: an aliphatic side-chain (G, A, V, L, I, P); a

hydroxyl group containing side-chain (S, T, Y); a sulfur atom containing side-chain (C, M); a carboxylic acid and amide containing side-chain (D, N, E, Q); a base containing side-chain (R, K, H); and an aromatic containing side-chain (H, F, Y, W). In addition, the following eight groups each contain amino acids that are accepted in the art as conservative substitutions for one another:

- [0074] 1) Alanine (A), Glycine (G);
- [0075] 2) Aspartic acid (D), Glutamic acid (E);
- [0076] 3) Asparagine (N), Glutamine (Q);
- [0077] 4) Arginine (R), Lysine (K);
- [0078] 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
- [0079] 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
- [0080] 7) Serine (S), Threonine (T); and
- [0081] 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins 1984).

[0082] Such conservatively modified peptides are also encompassed in peptides of the present invention. However, peptides of the present invention are not restricted thereto and can include non-conservative modifications, so long as the modified peptide retains the CTL-inducing ability of the original peptide. Furthermore, modified peptides do not exclude CTL inducible peptides derived from polymorphic variants, interspecies homologues, and alleles of CDCA1.

[0083] So long as a peptide retains the CTL-inducing ability of an original peptide, one can modify (i.e., substitute, delete, insert and/or add) a small number (for example, 1, 2 or several) or a small percentage of amino acids. Herein, the term “several” means 5 or fewer amino acids, for example, 4 or 3 or fewer. The percentage of amino acids to be modified is preferably 20% or less, more preferably 15% or less, even more preferably 10% or less or 1 to 5%.

[0084] When used in the context of immunotherapy, peptides of the present invention are presented on the surface of a cell or exosome, preferably as a complex with an HLA antigen. Therefore, it is preferable that the peptides of the present invention possess high binding affinity to the HLA antigen. To that end, the peptides can be modified by substitution, deletion, insertion, and/or addition of the amino acid residues to yield a modified peptide having improved binding affinity. Since the regularity of the sequences of peptides displayed by binding to HLA antigens is already known (Falk et al., Immunogenetics 1994, 40: 232-41; Chujoh, et al., Tissue Antigens 1998, 52: 501-9; Takiguchi et al., Tissue Antigens 2000, 55: 296-302.), modifications based on such regularity can be introduced into the peptides of the present invention.

[0085] For example, in peptides having binding affinity for HLA Class I, the second amino acid from the N terminus and the C-terminal amino acid are generally anchor residues involved in the binding to HLA Class I (Rainmensee HG, et al., Immunogenetics. 1995, 41(4): 178-228.). For example, for HLA-A01, aspartic acid and glutamic acid for the third amino acid from the N terminus, and tyrosine for the C-terminal amino acid are known as anchor residues with high binding affinity for HLA-A01. Further, in HLA-A01, there is auxiliary anchor residues at position 2 from the N terminus; and it is known that threonine and serine are preferred as the second amino acid from the N terminus (Kubo, R. T Journal of Immunology 1994, 152: 3913-24; Gambacorti-Passerini, C. Clinical Cancer Research 1997, 3: 675-83; Falk, K. Immunogenetics 1994, 40: 238-41). Thus,

to enhance the HLA-A01-binding affinity, there is a possibility that it is desirable to substitute the third amino acid from the N terminus with aspartic acid or glutamic acid, and/or to substitute the C-terminal amino acid with tyrosine. Further, there is a possibility that it is also desirable to substitute the second amino acid from the N terminus with threonine or serine. Thus, peptides with CTL-inducing ability, comprising an amino acid sequence in which, in the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37, the second amino acid from the N terminus is substituted with threonine or serine; the third amino acid from the N terminus is substituted with aspartic acid or glutamic acid; and/or the C-terminal amino acid is substituted with tyrosine are encompassed by the peptides of the present invention. In a preferred embodiment, the peptide of the present invention can be a peptide having CTL-inducing ability that consists of an amino acid sequence in which, in the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37, the second amino acid from the N terminus is substituted with threonine or serine; the third amino acid from the N terminus is substituted with aspartic acid or glutamic acid; and/or the C-terminal amino acid is substituted with tyrosine. That is, the peptides of the present invention encompass peptides having CTL-inducing ability, which comprise an amino acid sequence with one or more substitutions selected from (a) to (c) below in the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37:

[0086] (a) the second amino acid from the N terminus is substituted with threonine or serine;

[0087] (b) the third amino acid from the N terminus is substituted with aspartic acid or glutamic acid; and

[0088] (c) the C-terminal amino acid is substituted with tyrosine.

[0089] In a preferred embodiment, the peptide of the present invention may be a peptide having CTL-inducing ability that consists of an amino acid sequence in which one or more substitutions selected from (a) to (c) above are introduced into the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37. In the present invention, the preferred number of substitutions is 1, 2 or 3 substitutions selected from (a) to (c) above.

[0090] Furthermore, the peptide of the present invention may be a peptide having CTL-inducing ability, which comprises an amino acid sequence in which, in the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37, the third amino acid from the N terminus is substituted with aspartic acid or glutamic acid, and/or the C-terminal amino acid is substituted with tyrosine. Preferably, the peptide of the present invention may be a peptide having CTL-inducing ability, which consists of an amino acid sequence in which, in the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37, the third amino acid from the N terminus is substituted with aspartic acid or glutamic acid, and/or the C-terminal amino acid is substituted with tyrosine. That is, the peptide of the present invention may be a peptide having CTL-inducing ability, which comprises an amino acid sequence in which one or more substitutions selected from (a) and (b) below are introduced into the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37:

[0091] (a) the third amino acid from the N terminus is substituted with aspartic acid or glutamic acid; and

[0092] (b) the C-terminal amino acid is substituted with tyrosine.

[0093] In a preferred embodiment, the peptide of the present invention may be a peptide having CTL-inducing ability, which consists of an amino acid sequence in which one or more substitutions selected from (a) to (b) above are introduced into the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37.

[0094] Substitution(s) may be introduced into amino acid (s) not only at the anchor site(s), but also at a position(s) of potential T cell receptor (TCR) recognition site(s) of the peptides. Several research studies have demonstrated that a peptide that has amino acid substitutions, such as CAP1, p53₍₂₆₄₋₂₇₂₎, Her-2/neu₍₃₆₉₋₃₇₇₎ or gp100₍₂₀₉₋₂₁₇₎, may have equal to or better activity than that of the original peptide (Zaremba et al. Cancer Res. 1997, 57, 4570-7; T. K. Hoffmann et al. J Immunol. 2002, 168(3): 1338-47; S. O. Dionne et al. Cancer Immunol immunother. 2003, 52: 199-206; and S. O. Dionne et al. Cancer Immunology, Immunotherapy 2004, 53, 307-14).

[0095] The present invention also contemplates that one, two or several amino acids can be added to the N terminus and/or C terminus of the peptides of the present invention (for example, peptides consisting of the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37). Such modified peptides that retain CTL-inducing ability are also included in the present invention. For example, when a peptide in which one, two or several amino acids are added to the N terminus and/or C terminus of a peptide consisting of the amino acid sequence of SEQ ID NO: 8 is contacted with an APC(s), it is incorporated into the APC(s) and processed to become a peptide consisting of the amino acid sequence of SEQ ID NO: 8. It can then induce CTLs through presentation on the cell surface of an APC via the antigen presentation pathway. More specifically, peptides of the present invention can be peptides in which one, two or several amino acids are added to either or both of the N terminus and C terminus.

[0096] In yet another embodiment of the present invention, peptides consisting of an amino acid sequence which comprises one, two, or several amino acid substitutions in the amino acid sequence referenced by each SEQ ID NO and which further comprises one, two, or several amino acids added to either or both of the N-terminus and the C-terminus of the substitution-bearing amino acid sequence are provided.

[0097] When a peptide of the present invention comprises an amino acid substitution, a desired position for the substitution can be, for example, one, two, or three selected from position 2 from the N-terminus, position 3 from the N-terminus, and the C-terminus in the amino acid sequence referenced by SEQ ID NO: 1, 8, 10, 13, 25, 33 to 35 or 37 which is comprised in said peptide of the present invention.

[0098] However, when the amino acid sequence of a peptide is identical to a portion of the amino acid sequence of an endogenous or exogenous protein having a different function, side effects such as autoimmune disorders and/or allergic symptoms against specific substances may be induced. Therefore, it is preferable to perform homology searches using available databases to avoid situations in which the amino acid sequence of the peptide matches the amino acid sequence of another protein. When it becomes clear from the homology searches that no peptide exists with as few as 1 or 2 amino acid differences as compared to the

objective peptide, the objective peptide can be modified in order to increase its binding affinity with HLA antigens, and/or increase its CTL-inducing ability without danger of such side effects.

[0099] Peptides in which one, two or several amino acids of a peptide of the present invention are modified are predicted to be able to retain CTL-inducing ability of the original peptide; however, it is preferable to verify the CTL-inducing ability of the modified peptides. Herein, the "peptide having CTL-inducing ability (CTL inducibility)" refers to a peptide that induces CTLs through APCs stimulated with the peptide. "CTL induction" includes induction of differentiation into CTLs, induction of CTL activation, induction of CTL proliferation, induction of CTL's cytotoxic activity, induction of CTL-mediated dissolution of target cells, and induction of increase of IFN-gamma production of CTLs.

[0100] The CTL-inducing ability can be confirmed by inducing and stimulating APCs that retain an HLA antigen (for example, B lymphocytes, macrophages, and dendritic cells) with a peptide, and mixing them with CD8-positive T cells; and then measuring IFN-gamma released by CTLs against the target cells. For the APCs, human peripheral blood mononuclear leukocyte-derived dendritic cells can be preferably used. As a reaction system, transgenic animals generated to express an HLA antigen can be used. Alternatively, for example, the target cells may be radio-labelled with ⁵¹Cr or such, and the cytotoxic activity of the peptide-induced CTLs may be calculated from the radioactivity emitted from the target cells. Alternatively, in the presence of peptide-stimulated APCs, it is possible to evaluate the CTL-inducing ability by measuring the IFN-gamma produced and released by CTLs, and visualizing the inhibition zone on the media using anti-IFN-gamma monoclonal antibodies.

[0101] In addition to the modifications above, the peptides of the present invention can be linked to other peptides as long as the resultant linked peptide retains the CTL-inducing ability. An example of an appropriate peptide to be linked with the peptides of the present invention includes a TAA-derived CTL-inducing peptide. Further, the peptides of the present invention can also be linked with each other. Suitable linkers for use in linking peptides are known in the art, and for example, linkers such as AAY (P. M. Daftarian et al., J Trans Med. 2007, 5: 26), AAA, NKRRK (SEQ ID NO: 48) (R. P. M. Suttmuller et al., J Immunol. 2000, 165: 7308-15), or K (S. Ota et al., Can Res. 2002, 62, 1471-6; K. S. Kawamura et al., J Immunol. 2002, 168: 5709-15) can be used. Peptides can be linked in various arrangements (for example, catenulate, repeated, etc.), and one can also link three or more peptides.

[0102] The peptides of the present invention can also be linked to other substances as long as the resultant linked peptide retains the CTL-inducing ability. Examples of an appropriate substance to be linked with a peptide of the present invention include, for example, a peptide, a lipid, a sugar or sugar chain, an acetyl group, and a naturally-occurring or synthetic polymer. The peptides of the present invention can be modified by glycosylation, side-chain oxidation, phosphorylation or such, as long as their CTL-inducing ability is not impaired. One can also perform such types of modifications to confer additional functions (for example, targeting function and delivery function) or to stabilize the peptide.

[0103] For example, to increase the in vivo stability of a peptide, it is known in the art to introduce D-amino acids, amino acid mimetics or non-naturally occurring amino acids, and this concept may also be applied to peptides of the present invention. Peptide stability can be assayed by several methods. For example, stability can be tested by using a peptidase as well as various biological media such as human plasma and serum (see, e.g., Verhoef et al., *Eur J Drug Metab Phannacokin* 1986, 11: 291-302).

[0104] Further, as stated above, among the modified peptides in which one, two, or several amino acid residues have been substituted, deleted, inserted and/or added, those having equal to or higher activity as compared to original peptides can be screened for or selected. Thus, the present invention also provides methods of screening for or selecting modified peptides that have equal to or higher activity than that of the original peptide. Specifically, the present invention provides a method of screening for a peptide having CTL-inducing ability, wherein the method comprises the steps of:

[0105] (a) generating candidate sequences consisting of an amino acid sequence in which one, two, or several amino acid residues are substituted, deleted, inserted and/or added to the original amino acid sequence consisting of the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37;

[0106] (b) selecting from among the candidate sequences generated in (a), a candidate sequence that does not have a significant homology (sequence identity) with any known human gene product other than CDCA1;

[0107] (c) contacting a peptide consisting of the candidate sequence selected in (b) with APCs;

[0108] (d) contacting the APCs of (c) with CD8-positive T cells; and

[0109] (e) selecting a peptide that has an equal to or higher CTL-inducing ability than that of a peptide consisting of the original amino acid sequence.

[0110] In the present invention, all the peptides consisting of SEQ ID NO: 1, 8, 10, 13, 25, 33 to 35 or 37 have an HLA-A01-restrictive CTL-inducing activity. Therefore, in order to select ones with the CTL-inducing ability from their amino-acid-sequence-modified variants, the APCs in step (c) above are desirably cells having HLA-A01.

[0111] Herein, the peptide of the present invention is also described as a "CDCA1 peptide(s)" or a "CDCA1 polypeptide(s)".

III. Preparation of Peptides of the Present Invention

[0112] Well known techniques can be used to prepare peptides of the present invention. For example, recombinant DNA technology or chemical synthesis can be used to prepare peptides of the present invention. Peptides of the present invention can be synthesized individually, or as longer polypeptides including two or more peptides. Peptides of the present invention can be isolated from host cells or synthesis reaction products after they are produced in the host cells using recombinant DNA technology or after they are chemically synthesized. That is, peptides of the present invention can be purified or isolated so as not to substantially contain other host-cell proteins and fragments thereof, or any other chemical substances.

[0113] The peptides of the present invention may contain modifications, such as glycosylation, side chain oxidation,

or phosphorylation provided such modifications do not destroy the biological activity of the original peptide. Other illustrative modifications include incorporation of D-amino acids or other amino acid mimetics that may be used, for example, to increase the serum half life of the peptides.

[0114] A peptide of the present invention can be obtained through chemical synthesis based on the selected amino acid sequence. Examples of conventional peptide synthesis methods that can be adapted to the synthesis include the methods described in the documents below:

[0115] (i) *Peptide Synthesis*, Interscience, New York, 1966;

[0116] (ii) *The Proteins*, Vol. 2, Academic Press, New York, 1976;

[0117] (iii) "Peptide Synthesis" (in Japanese), Maruzen Co., 1975;

[0118] (iv) "Basics and Experiment of Peptide Synthesis" (in Japanese), Maruzen Co., 1985;

[0119] (v) "Development of Pharmaceuticals" (in Japanese), Continued Vol. 14 (peptide synthesis), Hirokawa, 1991;

[0120] (vi) WO99/67288; and

[0121] (vii) Barany G. & Merrifield R. B., *Peptides* Vol. 2, Solid Phase Peptide Synthesis, Academic Press, New York, 1980, 100-118.

[0122] Alternatively, the peptides of the present invention can be obtained by adapting any known genetic engineering methods for producing peptides (e.g., Morrison J, *J Bacteriology* 1977, 132: 349-51; Clark-Curtiss & Curtiss, Wu et al., *Methods in Enzymology* 1983, 101: 347-62). For example, first, a suitable vector harboring a polynucleotide encoding the peptide of the present invention in an expressible form (e.g., downstream of a regulatory sequence corresponding to a promoter sequence) is prepared and transformed into a suitable host cell. The host cell is then cultured to produce the peptide of the present invention. The peptide of the present invention can also be produced in vitro using an in vitro translation system.

IV. Polynucleotides

[0123] The present invention also provides a polynucleotide which encodes any of the peptides of the present invention. These include polynucleotides derived from the naturally occurring CDCA1 gene (e.g., GenBank Accession No. NM_145697 (SEQ ID NO: 44) or GenBank Accession No. NM_031423 (SEQ ID NO: 46)) as well as those having a conservatively modified nucleotide sequence thereof. Herein, the phrase "conservatively modified nucleotide sequence" refers to sequences which encode identical or essentially identical amino acid sequences. Due to the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG, and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described above without altering the encoded polypeptide. Such nucleic acid variations are "silent variations", which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a peptide also describes every possible silent variation of the nucleic acid. One of ordinary skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for

tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a peptide is implicitly described in each disclosed sequence.

[0124] The polynucleotide of the present invention can be composed of DNA, RNA, and derivatives thereof. A DNA is suitably composed of bases such as A, T, C, and G, and T is replaced by U in an RNA.

[0125] The polynucleotide of the present invention can encode multiple peptides of the present invention with or without intervening amino acid sequences in between. For example, the intervening amino acid sequence can provide a cleavage site (e.g., enzyme recognition sequence) of the polynucleotide or the translated peptides. Furthermore, the polynucleotide can include any additional sequences to the coding sequence encoding the peptide of the present invention. For example, the polynucleotide can be a recombinant polynucleotide that includes regulatory sequences required for the expression of the peptide or can be an expression vector (e.g., plasmid) with marker genes and such. In general, such recombinant polynucleotides can be prepared by the manipulation of polynucleotides through conventional recombinant techniques using, for example, polymerases and endonucleases.

[0126] Both recombinant and chemical synthesis techniques can be used to produce the polynucleotides of the present invention. For example, a polynucleotide can be produced by insertion into an appropriate vector, which can be expressed when transfected into a competent cell. Alternatively, a polynucleotide can be amplified using PCR techniques or expression in suitable hosts (see, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, 1989). Alternatively, a polynucleotide can be synthesized using the solid phase techniques, as described in Beaucage SL & Iyer RP, *Tetrahedron* 1992, 48: 2223-311; Matthes et al., *EMBO J* 1984, 3: 801-5.

V. Exosomes

[0127] The present invention further provides intracellular vesicles, referred to as exosomes, that present complexes formed between the peptides of the present invention and HLA antigens on their surface. Exosomes can be prepared, for example, using the methods detailed in JPH11-510507 and WO99/03499, and can be prepared using APCs obtained from patients who are subject to treatment and/or prevention (prophylaxis). The exosomes of the present invention can be inoculated as vaccines, in a fashion similar to the peptides of the present invention.

[0128] The type of the HLA antigens included in the above-described complexes must match that of the subject in need of treatment and/or prevention (prophylaxis). For example, HLA-A01 (for example, HLA-A*01:01) is an HLA allele highly frequently observed in Caucasians, and this HLA antigen type is considered to be suitable for treatment in Caucasian patients. Typically in clinical practice, it is possible to select an appropriate peptide that has a high level of binding affinity for a specific HLA antigen or that has CTL-inducing ability by antigen presentation mediated by a specific HLA antigen, by studying in advance the HLA antigen type of the patient in need of treatment.

[0129] The exosomes of the present invention present on their surface a complex of a peptide of the present invention and HLA-A01. When the HLA that forms a complex with a

peptide of the present invention is HLA-A01, the peptide of the present invention is preferably a peptide having the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37 or a modified peptide thereof, and more preferably a peptide consisting of the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37 or a modified peptide thereof.

VI. Antigen-Presenting Cells (APCs)

[0130] The present invention further provides APCs that present on their surface complexes formed between HLA antigens and peptides of the present invention. Alternatively, the present invention provides APCs having on their cell surface complexes formed between HLA antigens and peptides of the present invention. The APCs of the present invention can be isolated APCs. When used in the context of cells (APCs, CTLs, etc.), the term “isolated” means that the cells are separated from another type of cells. The APCs of the present invention may be APCs induced from APCs derived from the patient to be subjected to treatment and/or prevention (prophylaxis), and can be administered as a vaccine by themselves or in combination with other drugs including a peptide(s), an exosome(s) or a CTL(s) of the present invention.

[0131] The APCs of the present invention are not limited to specific types of cells, and include cells known to present proteinaceous antigens on their cell surface so as to be recognized by lymphocytes, for example, dendritic cells (DCs), Langerhans cells, macrophages, B cells, and activated T cells. Since DC is a representative APC that has the strongest CTL-inducing activity among APCs, DCs can be preferably used as the APCs of the present invention.

[0132] For example, APCs of the present invention can be obtained by inducing DCs from peripheral blood monocytes and then stimulating them in vitro, ex vivo or in vivo with the peptides of the present invention. When the peptide of the present invention is administered to a subject, APCs presenting the peptide of the present invention are induced in the body of the subject. Therefore, after the peptides of the present invention are administered to a subject, the APCs of the present invention can be obtained by collecting APCs from the subject. Alternatively, the APCs of the present invention can be obtained by contacting APCs collected from a subject with a peptide of the present invention.

[0133] In order to induce an immune response against CDCA1-expressing cancer cells in a subject, the APCs of the present invention can be administered to the subject by themselves or in combination with other drugs including peptide(s), exosome(s) or CTL(s) of the present invention. For example, the ex vivo administration can comprise the following steps of:

[0134] (a) collecting APCs from a first subject;

[0135] (b) contacting the APCs of step (a) with a peptide; and

[0136] (c) administering the APCs of step (b) to a second subject.

[0137] The first subject and the second subject may be the same individual, or may be different individuals. When the first subject and the second subject are different individuals, it is preferable that the HLAs of the first subject and the second subject are of the same type. The APC obtained in step (b) above can be a vaccine for cancer treatment and/or prevention (prophylaxis).

[0138] The APCs of the present invention obtained by a method such as described above have CTL-inducing ability. The term “CTL-inducing ability (CTL inducibility)” used in the context of an APC(s) refers to the ability of the APC to be able to induce a CTL(s) when placed in contact with a CD8-positive T cell(s). The CTL(s) induced by the APC of the present invention is a CTL(s) specific to CDCA1 and demonstrates a specific cytotoxic activity against CDCA1-expressing cells.

[0139] In addition to the above-described methods, the APCs of the present invention can be prepared by introducing a polynucleotide encoding a peptide of the present invention into APCs in vitro. The polynucleotide to be introduced can be in the form of DNA or RNA. The method of introduction is not particularly limited, and examples thereof include various methods conventionally performed in the art such as lipofection, electroporation and the calcium phosphate method. More specifically, methods described in Cancer Res 1996, 56: 5672-7; J Immunol 1998, 161: 5607-13; J Exp Med 1996, 184: 465-72; and JP2000-509281 can be used. By introducing a polynucleotide encoding a peptide of the present invention into an APC, the polynucleotide is transcribed and translated in the cell, and then the produced peptide is processed by MHC Class I and proceeds through a presentation pathway to present the peptide of the present invention on the cell surface of the APC.

[0140] In a preferred embodiment, the APC of the present invention is an APC that presents on its cell surface a complex formed between HLA-A01 (more preferably HLA-A*01:01) and a peptide of the present invention. When the HLA that forms a complex with a peptide of the present invention is HLA-A01, the peptide of the present invention is preferably a peptide having the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37 or a modified peptide thereof, and more preferably a peptide consisting of the amino sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37.

[0141] The APC(s) of the present invention is preferably an APC(s) induced by a method comprising a step described (a) or (b) below:

[0142] (a) contacting an APC(s) expressing HLA-A01 (more preferably HLA-A*01:01) with a peptide of the present invention; or

[0143] (b) introducing a polynucleotide encoding a peptide of the present invention into an APC(s) expressing HLA-A01 (more preferably HLA-A*01:01).

[0144] The peptide of the present invention to be contacted with the HLA-A01-expressing APC(s) is preferably a peptide having the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37 or a modified peptide thereof, and more preferably a peptide consisting of the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37.

[0145] The present invention provides use of a peptide of the present invention for the manufacture of a pharmaceutical composition that induces an APC(s) having CTL-inducing ability. In addition, the present invention provides a method or process of manufacturing a pharmaceutical composition that induces an APC(s) having CTL-inducing ability. Further, the present invention provides a peptide of the present invention for inducing an APC(s) having CTL-inducing ability.

VII. Cytotoxic T Lymphocytes (CTLs)

[0146] The CTL induced by a peptide of the present invention can be used as a vaccine in a similar manner to the peptide of the present invention since it enhances an immune response targeting CDCA1-expressing cancer cell in vivo. Thus, the present invention provides CTLs that are induced or activated by a peptide of the present invention. The CTLs of the present invention are CTLs that target a peptide of the present invention, and are capable of binding to a complex of a peptide of the present invention and an HLA antigen. Binding of a CTL to the complex is mediated via a T cell receptor (TCR) present on the cell surface of the CTL. The CTLs of the present invention can be isolated CTLs.

[0147] The CTLs of the present invention can be obtained by (1) administering a peptide of the present invention to a subject, (2) stimulating APCs and CD8-positive T cells, or peripheral blood mononuclear cells (PBMCs) derived from a subject with a peptide of the present invention in vitro, (3) contacting in vitro CD8-positive T cells or PBMCs with APCs or exosomes that present on their surface a complex of an HLA antigen and a peptide of the present invention, or (4) introducing into CD8-positive T cells a vector comprising a polynucleotide encoding each subunit of a T cell receptor (TCR) capable of binding to a peptide of the present invention presented on cell surface via an HLA antigen. The exosomes and APCs used in the method of (2) or (3) above can be prepared by methods described in the “V. Exosomes” and “VI. Antigen-presenting cells (APCs)” sections, respectively, and the details of the method of (4) above will be described in the “VIII. T cell receptors (TCRs)” section.

[0148] The CTLs of the present invention can be administered by themselves to patients who are subject to treatment and/or prevention (prophylaxis), or in combination with other drugs including peptide(s), APC(s) or exosome(s) of the present invention for the purpose of regulating effects. Further, the CTLs of the present invention can be CTLs induced from CD8-positive T cells derived from the patients who are subject to administration of the CTLs. The CTLs of the present invention act specifically on target cells that present the peptides of the present invention, for example, the same peptides used to induce the CTLs of the present invention. The target cells may be cells that endogenously express CDCA1, such as cancer cells, or cells transfected with the CDCA1 gene. Cells that present a peptide of the present invention on their cell surface due to stimulation by the peptide can become a target of attack by the CTLs of the present invention. The cells targeted by the CTLs of the present invention are preferably cells that are positive for HLA-A01 (more preferably HLA-A*01:01).

[0149] In a preferred embodiment, the CTLs of the present invention target specifically cells that express both HLA-A01 (more preferably HLA-A*01:01) and CDCA1. Herein, that the CTL “targets” cells refers to CTL recognition of cells that present on their cell surface a complex of HLA and a peptide of the present invention and demonstration of a cytotoxic activity against the cells. Further, “specifically target” refers to that the CTLs demonstrate a cytotoxic activity against those cells, but do not show a damaging activity to other cells. The expression “recognize cells” used in the context of CTLs refers to binding to a complex of HLA and a peptide of the present invention presented on cell surface via its TCR, and demonstrating a specific cytotoxic activity against the cell. Therefore, the CTLs of the present invention are preferably CTLs that can bind via TCR to a

complex formed between HLA-A01 (more preferably HLA-A*01:01) and a peptide of the present invention presented on cell surface.

[0150] Furthermore, the CTLs of the present invention are preferably CTLs induced by a method comprising a step described in (a) or (b) below:

[0151] (a) contacting in vitro CD8-positive T cells with APCs or exosomes that present on their surface a complex of HLA-A01 (more preferably HLA-A*01:01) and a peptide of the present invention; or

[0152] (b) introducing into CD8-positive T cells a polynucleotide encoding each subunit of a TCR capable of binding to a peptide of the present invention presented on cell surface by HLA-A01 (more preferably HLA-A*01:01).

VIII. T Cell Receptors (TCRs)

[0153] The present invention also provides compositions comprising a polynucleotide encoding each subunit of a TCR capable of binding to a peptide of the present invention presented on cell surface by an HLA antigen, and methods of using the same. The polynucleotide confers CD8-positive T cells with specificity against CDCA1-expressing cancer cells through expression of a TCR on cell surface capable of binding to a peptide of the present invention presented on cell surface by an HLA antigen. Polynucleotides encoding an alpha chain(s) and a beta chain(s) can be identified as the TCR subunit of the CTL induced by a peptide of the present invention by using known methods in the art (WO2007/032255 and Morgan et al., J Immunol, 2003, 171, 3288). For example, PCR methods are preferred for TCR analysis. PCR primers for analysis may be, for example, 5'-R Primer (5'-gtctaccaggcatcgcgtcat-3') (SEQ ID NO: 40) as a 5' side primer; and 3-TRa-C Primer (5'-tcagctggaccacagccgcagcgt-3') (SEQ ID NO: 41) specific to TCR-alpha-chain C-region, 3-TRb-C1 Primer (5'-tcagaaatcttctcttgac-3') (SEQ ID NO: 42) specific to TCR-beta-chain C1-region, or 3-TRb-C2 Primer (5'-ctagcctctggaatccttctctt-3') (SEQ ID NO: 43) specific to TCR-beta-chain C2-region as 3' side primers, but are not limited thereto. The TCRs formed by introducing the identified polynucleotides into CD8-positive T cells can bind with high binding affinity to the target cells that present a peptide of the present invention, and mediates efficient killing of the target cells presenting a peptide of the present invention in vivo and in vitro.

[0154] A polynucleotide encoding each TCR subunit can be incorporated into an appropriate vector, for example, retrovirus vector. These vectors are well known in the art. The polynucleotide or a vector comprising thereof in an expressible form can be introduced into a CD8-positive T cell, for example, a CD8-positive T cell derived from a patient. The present invention provides off-the-shelf compositions that allow rapid and easy production of modified T cells that have superior cancer cell-killing properties by rapid modification of the patient's own T cells (or T cells derived from another subject).

[0155] Herein, a specific TCR is a TCR that can confer a specific cytotoxic activity against target cells by specifically recognizing a complex of a peptide of the present invention and an HLA antigen presented on the surface of the target cell when the TCR is present on the surface of a CD8-positive T cell. Specific recognition of the above-described complex can be confirmed by any known method, and preferable examples thereof include HLA multimer staining

analysis using HLA molecules and peptides of the present invention and ELISPOT assay methods. Specific TCR-mediated recognition of target cell by T cell introduced with the above-described polynucleotide and signal transduction in the cell can be confirmed by carrying out an ELISPOT assay. When the above-described TCR is present on the surface of a CD8-positive T cell, whether the TCR can confer a target cell-specific cytotoxic activity against the CD8-positive T cell can also be confirmed by known methods. Preferable methods include, for example, measuring the cytotoxic activity against target cells by a chrome release assay method or such.

[0156] The present invention provides, in the context of HLA-A01, CTLs prepared by transforming CD8-positive T cells with a polynucleotide encoding each subunit of TCR that binds to, for example, a peptide having the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37.

[0157] The transformed CTLs are capable of homing in vivo and may be propagated by a well-known in vitro culturing method (for example, Kawakami et al., J Immunol., 1989, 142, 3452-61). The CTLs of the present invention can be used to form an immunogenic composition useful for disease treatment or prevention (prophylaxis) in a patient in need of treatment or prevention (prophylaxis) (the contents are incorporated herein for reference WO2006/031221).

IX. Pharmaceutical Compositions

[0158] The present invention further provides compositions or pharmaceutical compositions, comprising at least one active ingredient selected from below:

[0159] (a) a peptide of the present invention;

[0160] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0161] (c) an APC of the present invention;

[0162] (d) an exosome of the present invention; and

[0163] (e) a CTL of the present invention.

[0164] The pharmaceutical compositions of the present invention can comprise as needed a carrier(s), an excipient (s) or such commonly used in pharmaceuticals without particular limitations, in addition to the active ingredient(s) described above. An example of a carrier that can be used in a pharmaceutical composition of the present invention includes sterilized water, physiological saline, phosphate buffer, culture fluid and such. Therefore, the present invention also provides pharmaceutical compositions, comprising at least one active ingredient selected from (a) to (e) below and a pharmaceutically acceptable carrier:

[0165] (a) a peptide of the present invention;

[0166] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0167] (c) an APC of the present invention;

[0168] (d) an exosome of the present invention; and

[0169] (e) a CTL of the present invention.

[0170] Further, the pharmaceutical compositions of the present invention can comprise, as needed, stabilizers, suspensions, preservatives, surfactants, solubilizing agents, pH adjusters, aggregation inhibitors and such.

[0171] The CDCA1 expression significantly up-regulates in cancer cells compared with normal tissues. Thus, a peptide of the present invention or a polynucleotide encoding the peptide can be used to treat and/or prevent cancer, and/or prevent postoperative recurrence thereof. Therefore,

the present invention provides pharmaceutical compositions for treating and/or preventing cancer, and/or preventing postoperative recurrence thereof, comprising one or more types of peptides or polynucleotides of the present invention as an active ingredient. Alternatively, the peptides of the present invention can be made to be presented on the surface of exosomes or APCs for use as pharmaceutical compositions. In addition, CTLs of the present invention targeting any one of the peptides of the present invention can also be used as an active ingredient of the pharmaceutical compositions of the present invention. The pharmaceutical compositions of the present invention may comprise a therapeutically effective amount or a pharmaceutically effective amount of the above-described active ingredient.

[0172] The pharmaceutical compositions of the present invention may also be used as a vaccine. In the context of the present invention, the term “vaccine” (also called “immunogenic composition”) refers to a composition that has a function of inducing an immune response that leads to antitumor action when inoculated into an animal. Thus, a pharmaceutical composition of the present invention can be used to induce an immune response that leads to antitumor action. The immune response induced by a peptide, a polynucleotide, an APC, a CTL and a pharmaceutical composition of the present invention is not particularly limited as long as it is an immune response that leads to antitumor action, and examples include induction of cancer cell-specific CTLs and induction of cancer cell-specific cytotoxic activity.

[0173] The pharmaceutical compositions of the present invention can be used to treat and/or prevent cancer, and/or prevent postoperative recurrence thereof in human subjects or patients. The pharmaceutical compositions of the present invention can be used preferably to a subject positive for HLA-A01. Further, the pharmaceutical compositions of the present invention can be used preferably to treat and/or prevent cancers expressing HLA A01 and CDCA1, and/or prevent postoperative recurrence thereof.

[0174] In another embodiment, the present invention provides use of an active ingredient selected from below in the manufacture of a pharmaceutical composition for treating or preventing cancer expressing HLA-A01 and CDCA1:

[0175] (a) a peptide of the present invention;

[0176] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0177] (c) an APC that presents a peptide of the present invention on its surface;

[0178] (d) an exosome that presents a peptide of the present invention on its surface; and

[0179] (e) a CTL of the present invention.

[0180] Alternatively, the present invention further provides an active ingredient selected from below for use in treating or preventing cancer expressing HLA-A01 and CDCA1:

[0181] (a) a peptide of the present invention;

[0182] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0183] (c) an APC that presents a peptide of the present invention on its surface;

[0184] (d) an exosome that presents a peptide of the present invention on its surface; and

[0185] (e) a CTL of the present invention.

[0186] Alternatively, the present invention further provides a method or process for manufacturing a pharmaceu-

tical composition for treating or preventing cancer expressing HLA-A01 and CDCA1, wherein the method or process comprises a step of formulating at least one active ingredient selected from below with a pharmaceutically or physiologically acceptable carrier:

[0187] (a) a peptide of the present invention;

[0188] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0189] (c) an APC that presents a peptide of the present invention on its surface;

[0190] (d) an exosome that presents a peptide of the present invention on its surface; and

[0191] (e) a CTL of the present invention.

[0192] In another embodiment, the present invention further provides a method or process for manufacturing a pharmaceutical composition for treating or preventing cancer expressing HLA-A01 and CDCA1, wherein the method or process comprises a step of mixing an active ingredient selected from below with a pharmaceutically or physiologically acceptable carrier:

[0193] (a) a peptide of the present invention;

[0194] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0195] (c) an APC that presents a peptide of the present invention on its surface;

[0196] (d) an exosome that presents a peptide of the present invention on its surface; and

[0197] (e) a CTL of the present invention.

[0198] In another embodiment, the present invention further provides a method for treating or preventing cancer expressing HLA-A01 and CDCA1, which comprises a step of administering to a subject at least one active ingredient selected from below:

[0199] (a) a peptide of the present invention;

[0200] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0201] (c) an APC that presents a peptide of the present invention on its surface;

[0202] (d) an exosome that presents a peptide of the present invention on its surface; and

[0203] (e) a CTL of the present invention.

[0204] In the present invention, peptides having the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37 are identified as HLA-A01-restricted epitope peptides that can induce a potent and specific immune response. Therefore, pharmaceutical compositions of the present invention comprising at least one peptide having the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37 are suitable particularly for administration to a subject having HLA-A01 (for example, HLA-A*01:01) as an HLA antigen. The same applies to pharmaceutical compositions comprising a polynucleotide encoding any of these peptides (i.e., polynucleotides of the present invention), an APC or exosome that presents these peptides (i.e., APCs or exosomes of the present invention), or a CTL targeting these peptides (i.e., CTLs of the present invention). That is, pharmaceutical compositions comprising an active ingredient in association with a peptide having the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37 are suitable for administration to subjects having HLA-A01 (i.e., HLA-A01-positive subjects). In a more preferred embodiment, the pharmaceutical composition of the present

invention is a pharmaceutical composition that comprises a peptide having the amino acid sequence of SEQ ID NO: 8.

[0205] Cancers to be treated and/or prevented by pharmaceutical compositions of the present invention are not particularly limited as long as they are cancers that express CDCA1, and include various cancers, bladder cancer, breast cancer, cervical cancer, cholangiocellular cancer, chronic myeloid leukemia (CML), esophagus cancer, gastric cancer, non-small-cell lung cancer, lymphoma, osteosarcoma, prostate cancer, kidney cancer, small-cell lung cancer, head and neck cancer, soft tissue tumor, colon cancer and such.

[0206] In addition to the active ingredients described above, the pharmaceutical compositions of the present invention can comprise the other peptides that have the ability to induce CTLs against cancer cells (for example, the other TAA-derived CTL-inducing peptides), the other polynucleotides encoding the other peptides, the other cells that present the other peptides, or such.

[0207] The pharmaceutical compositions of the present invention may also optionally comprise the other therapeutic substances as an active ingredient, as long as they do not inhibit the anti-tumor effects of the above-described active ingredients such as peptides of the present invention. For example, the pharmaceutical compositions of the present invention may optionally comprise anti-inflammatory compositions, analgesics, chemotherapeutics and the like. In addition to including the other therapeutic substances to a pharmaceutical composition of the present invention itself, one can also administer the pharmaceutical composition of the present invention sequentially or concurrently with one or more other pharmaceutical compositions. The dose of the pharmaceutical composition of the present invention and the other pharmaceutical compositions depend on, for example, the type of pharmaceutical composition used and the disease being treated, as well as the scheduling and routes of administration.

[0208] It should be understood that in consideration of the formulation type, the pharmaceutical composition of the present invention may include other components conventional in the art, in addition to the ingredients specifically mentioned herein.

[0209] The present invention also provides articles of manufacture or kits that comprise a pharmaceutical composition of the present invention. The articles of manufacture or kits of the present invention can include a container that houses the pharmaceutical composition of the present invention. An example of an appropriate container includes a bottle, a vial or a test tube, but is not limited thereto. The container may be formed of various materials such as glass or plastic. A label may be attached to the container, and the disease or disease state to which the pharmaceutical composition of the present invention should be used may be described in the label. The label may also indicate directions for administration and such.

[0210] The articles of manufacture or kits of the present invention may further comprise a second container that houses pharmaceutically acceptable diluents optionally, in addition to the container that houses the pharmaceutical composition of the present invention. The articles of manufacture or kits of the present invention may further comprise the other materials desirable from a commercial standpoint and the user's perspective, such as the other buffers, diluents, filters, injection needles, syringes, package inserts with instructions for use.

[0211] As needed, the pharmaceutical composition of the present invention can be provided in a pack or dispenser device that can contain one or more units of dosage forms containing active ingredients. The pack can include, for example, a metallic foil or a plastic foil such as a blister pack. Instructions for administration can be attached to the pack or dispenser device.

(1) Pharmaceutical Compositions Comprising Peptide(s) as an Active Ingredient

[0212] The pharmaceutical composition comprising a peptide of the present invention can be formulated by conventional formulation methods as needed. The pharmaceutical compositions of the present invention may comprise as needed in addition to the peptide of the present invention, carriers, excipients and such commonly used in pharmaceuticals without particular limitations. Examples of carriers that can be used in pharmaceutical compositions of the present invention include sterilized water (for example, water for injection), physiological saline, phosphate buffer, phosphate buffered saline, Tris buffered saline, 0.3% glycine, culture fluid, and such. Further, the pharmaceutical compositions of the present invention may comprise as needed stabilizers, suspensions, preservatives, surfactants, solubilizing agents, pH adjusters, aggregation inhibitors, and such. The pharmaceutical compositions of the present invention can induce specific immunity against CDCA1-expressing cancer cells, and thus can be applied for the purpose of cancer treatment or prevention (prophylaxis).

[0213] For example, the pharmaceutical compositions of the present invention can be prepared by dissolving in pharmaceutically or physiologically acceptable water-soluble carriers such as sterilized water (for example, water for injection), physiological saline, phosphate buffer, phosphate buffered saline, and Tris buffered saline and adding, as needed, stabilizers, suspensions, preservatives, surfactants, solubilizing agents, pH adjusters, aggregation inhibitors and such, and then sterilizing the peptide solution. The method of sterilizing a peptide solution is not particularly limited, and is preferably carried out by filtration sterilization. Filtration sterilization can be performed using, for example, a filtration sterilization filter of 0.22 micro-m or less in pore diameter. The filtration-sterilized peptide solution can be administered to a subject, for example, as an injection, without being limited thereto. The pharmaceutical compositions of the present invention may be prepared as a freeze-dried formulation by freeze-drying the above-described peptide solution. The freeze-dried formulation can be prepared by filling the peptide solution prepared as described above into an appropriate container such as an ampule, a vial or a plastic container, followed by freeze drying and encapsulation into the container with a wash-sterilized rubber plug or such after pressure recovery. The freeze-dried formulation can be administered to a subject after it is re-dissolved in pharmaceutically or physiologically acceptable water-soluble carriers such as sterilized water (for example, water for injection), physiological saline, phosphate buffer, phosphate buffered saline, Tris buffered saline and such before administration. Preferred examples of pharmaceutical compositions of the present invention include injections of such a filtration-sterilized peptide solution, and freeze-dried formulations that result from freeze-drying the peptide solution. The present invention further encompasses kits comprising such a freeze-dried formula-

tion and re-dissolving solution. The present invention also encompasses kits comprising a container that houses the freeze-dried formulation, which is a pharmaceutical composition of the present invention, and a container that stores a re-dissolving solution thereof.

[0214] The pharmaceutical compositions of the present invention can comprise a combination of two or more types of the peptides of the present invention. The combination of peptides can take a cocktail form of mixed peptides, or can be conjugated with each other using standard techniques. For example, peptides can be chemically linked or expressed as single fusion polypeptide sequences. By administering a peptide of the present invention, the peptide is presented on APCs by an HLA antigen at a high density, and then subsequently CTLs that react specifically to a complex formed between the presented peptide and the HLA antigen are induced. Alternatively, APCs (for example, DCs) are removed from a subject, and subsequently stimulated with peptides of the present invention to obtain APCs that present any of the peptides of the present invention on their cell surface. These APCs are re-administered to a subject to induce CTLs in the subject, and as a result, the aggressiveness towards CDCA1-expressing cancer cells can be increased.

[0215] The pharmaceutical compositions of the present invention may also comprise an adjuvant known for effectively establishing cellular immunity. An adjuvant refers to a compound that enhances the immune response against an antigen that has immunological activity when administered together (or successively) with the antigen. Known adjuvants described in literatures, for example, Clin Microbiol Rev 1994, 7: 277-89, can be used. Examples of a suitable adjuvant include aluminum salts (aluminum phosphate, aluminum hydroxide, aluminum oxyhydroxide and such), alum, cholera toxin, Salmonella toxin,

[0216] Incomplete Freund's adjuvant (IFA), Complete Freund's adjuvant (CFA), ISCOMatrix, GM-CSF and other immunostimulatory cytokines, oligodeoxynucleotide containing the CpG motif (CpG7909 and such), oil-in-water emulsions, Saponin or its derivatives (QS21 and such), lipopolysaccharide such as Lipid A or its derivatives (MPL, RC529, GLA, E6020 and such), lipopeptides, lactoferrin, flagellin, double-stranded RNA or its derivatives (poli IC and such), bacterial DNA, imidazoquinolines (Imiquimod, R848 and such), C-type lectin ligand (trehalose-6,6'-dibehenate (TDB) and such), CD1d ligand (alpha-galactosylceramide and such), squalene emulsions (MF59, AS03, AF03 and such), PLGA, and such, without being limited thereto. These adjuvants can usually be mixed with an antigen having immunological activity (i.e., the peptide of the present invention) in an amount effective for enhancing or strengthening the antigen's immunogenicity. The adjuvant may be contained in another container separate from the pharmaceutical composition comprising a peptide of the present invention in the kits comprising the pharmaceutical composition of the present invention. In this case, the adjuvant and the pharmaceutical composition may be administered to a subject in succession, or mixed together immediately before administration to a subject. Such kits comprising a pharmaceutical composition comprising a peptide of the present invention and an adjuvant are also provided by the present invention. When the pharmaceutical composition of the present invention is a freeze-dried formulation, the kit can further comprise a re-dissolving solu-

tion. Further, the present invention provides kits comprising a container that houses a pharmaceutical composition of the present invention and a container that stores an adjuvant. The kit can further comprise as needed a container that stores the re-dissolving solution.

[0217] When an oil adjuvant is used as an adjuvant, the pharmaceutical composition of the present invention may be prepared as an emulsion. Emulsions can be prepared, for example, by mixing and stirring the peptide solution prepared as described above and an oil adjuvant. The peptide solution may be one that has been re-dissolved after freeze-drying. The emulsion may be either of the W/O-type emulsion and O/W-type emulsion, and the W/O-type emulsion is preferred for obtaining a high immune response-enhancing effect. IFA can be preferably used as an oil adjuvant, without being limited thereto. Preparation of an emulsion can be carried out immediately before administration to a subject, and in this case, the pharmaceutical composition of the present invention may be provided as a kit comprising the peptide solution of the present invention and an oil adjuvant. When the pharmaceutical composition of the present invention is a freeze-dried formulation, the kit can further comprise a re-dissolving solution.

[0218] Further, the pharmaceutical composition of the present invention may be a liposome formulation within which a peptide of the present invention is encapsulated, a granular formulation in which a peptide is bound to beads with several micrometers in diameter, or a formulation in which a lipid is bound to a peptide.

[0219] In another embodiment of the present invention, the peptide of the present invention may also be administered in the form of a pharmaceutically acceptable salt. Preferred examples of salts include salts with alkali metals (lithium, potassium, sodium and such), salts with alkaline-earth metals (calcium, magnesium and such), salts with other metals (copper, iron, zinc, manganese and such), salts with organic bases, salts with amines, salts with organic acids (acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid and such), and salts with inorganic acids (hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid, nitric acid and such). Therefore, pharmaceutical compositions comprising a pharmaceutically acceptable salt of a peptide of the present invention are also encompassed by the present invention. Further, the "peptide of the present invention" also encompasses, in addition to the free peptide, pharmaceutically acceptable salts thereof.

[0220] In some embodiments, the pharmaceutical compositions of the present invention may further include a component which primes CTLs. Lipids have been identified as substances capable of priming CTLs in vivo against viral antigens. For example, palmitic acid residues can be attached to the epsilon- and alpha-amino groups of a lysine residue and then linked to a peptide of the present invention. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant. As another example of lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinyl-seryl-serine (P3CSS) can be used to prime CTLs when covalently attached to an appropriate peptide (see, e.g., Deres et al., Nature 1989, 342: 561-4).

[0221] Examples of methods for administering the peptides or pharmaceutical compositions of the present invention include oral, epidermal, subcutaneous, intramuscular, intraosseous, peritoneal, and intravenous injections, as well as systemic administration or local administration to the vicinity of the targeted sites, but are not limited thereto. A preferred administration method includes subcutaneous injection to the vicinity of lymph nodes such as the armpit or groin. The administration can be performed by single administration or boosted by multiple administrations. The peptides of the present invention can be administered to a subject in a therapeutically or pharmaceutically effective amount for treating cancer or in a therapeutically or pharmaceutically effective amount for inducing immunity (more specifically CTLs) against CDCA1-expressing cancer cells. The dose of the peptides of the present invention can be appropriately adjusted according to the disease to be treated, the patient's age and weight, the method of administration and such. For each of the peptides of the present invention, the dose is usually 0.001 mg-1000 mg, for example, 0.01 mg-100 mg, for example, 0.1 mg-30 mg, for example, 0.1 mg-10 mg, for example, 0.5 mg-5 mg. The dosing interval can be once every several days to several months, and for example, the dosing can be done in a once-per-week interval. A skilled artisan can appropriately select a suitable dosage.

[0222] In a preferred embodiment, the pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a peptide of the present invention and a pharmaceutically or physiologically acceptable carrier. In another embodiment, the pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a peptide of the present invention, a pharmaceutically or physiologically acceptable cancer, and an adjuvant. The pharmaceutical compositions of the present invention can comprise 0.001 mg-1000 mg, preferably 0.01 mg-100 mg, more preferably 0.1 mg-30 mg, even more preferably 0.1 mg-10 mg, for example, 0.5 mg-5 mg of a peptide of the present invention.

[0223] When a pharmaceutical composition of the present invention is an injection, it can comprise a peptide of the present invention at a concentration of 0.001 mg/ml-1000 mg/ml, preferably 0.01 mg/ml-100 mg/ml, more preferably 0.1 mg/ml-30 mg/ml, even more preferably 0.1 mg/ml-10 mg/ml, for example, 0.5 mg/ml-5 mg/ml. In this case, for example, 0.1 to 5 ml, preferably 0.5 ml to 2 ml of the pharmaceutical composition of the present invention can be administered to a subject by injection.

[0224] Further, the present invention provides methods of treating and/or preventing cancer and/or preventing postoperative recurrence thereof, which comprise administering to a subject a therapeutically effective amount of a peptide of the present invention or a pharmaceutical composition of the present invention. As described above, the peptides of the present invention can be administered to a subject in a single dose of usually 0.001 mg-1000 mg, for example, 0.01 mg-100 mg, for example, 0.1 mg-30 mg, for example, 0.1 mg-10 mg, or for example, 0.5 mg-5 mg. In a preferred embodiment, the peptides of the present invention are administered to a subject together with an adjuvant. Further, the dosing interval can be once every several days to several months, preferably once every several days to every month, for example, once every week or once every two weeks.

[0225] The CDCA1 peptides of the invention carry an HLA-A01-restrictive CTL-inducing activity. Accordingly, their therapeutic effect is also effective in HLA-A01-positive subjects. Thus, in a preferred embodiment of the present invention, an HLA-A01-positive subject can be selected in advance, before the administration of a CDCA1 peptide of the present invention. Furthermore, since the therapeutic effect of the CDCA1 of the present invention is CDCA1-specific, it is a desirable condition that cancer in a subject is expressing CDCA1. That is, the methods of treating cancer of the present invention can comprise the step of selecting an HLA-A01-positive subject and the step of selecting a subject having cancer expressing CDCA1, before the administration of the CDCA1 peptide.

(2) Pharmaceutical Compositions Containing Polynucleotides as the Active Ingredient

[0226] The pharmaceutical compositions of the present invention can also contain polynucleotides encoding the peptides of the present invention in an expressible form. Herein, the phrase "in an expressible form" means that the polynucleotide, when introduced into a cell, will be expressed as a peptide of the present invention. In an exemplified embodiment, the sequence of the polynucleotide of the present invention includes regulatory elements necessary for expression of the peptide of the present invention. The polynucleotide(s) of the present invention can be equipped with a sequence necessary to achieve stable insertion into the genome of the target cell (see, e.g., Thomas K R & Capecchi M R, Cell 1987, 51: 503-12 for a description of homologous recombination cassette vectors). See, e.g., Wolff et al., Science 1990, 247: 1465-8; U.S. Pat. Nos. 5,580,859, 5,589,466, 5,804,566, 5,739,118, 5,736,524, 5,679,647; and WO98/04720. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivacaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (see, e.g., U.S. Pat. No. 5,922,687).

[0227] The peptides of the present invention can also be expressed by viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as vaccinia or fowlpox. For example, as a vector to express the peptide of the present invention, vaccinia virus can be used. Upon introduction into a host, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits an immune response. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Pat. No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover et al., Nature 1991, 351: 456-60. A wide variety of other vectors useful for therapeutic administration or immunization, e.g., adeno and adeno-associated virus vectors, retroviral vectors, Salmonella typhi vectors, detoxified anthrax toxin vectors, and the like, will be apparent. See, e.g., Shata et al., Mol Med Today 2000, 6: 66-71; Shedlock et al., J Leukoc Biol 2000, 68: 793-806; Hipp et al., In Vivo 2000, 14: 571-85.

[0228] Delivery of a polynucleotide of the present invention into a patient can be either direct, in which case the patient can be directly exposed to a vector harboring the polynucleotide of the present invention, or indirect, in which case, cells are first transformed with the vector harboring the polynucleotide of the present invention in vitro, then the

cells are transplanted into the patient. These two approaches are known, respectively, as *in vivo* and *ex vivo* gene therapies.

[0229] For general reviews of the methods of gene therapy, see Goldspiel et al., *Clinical Pharmacy* 1993, 12: 488-505; Wu and Wu, *Biotherapy* 1991, 3: 87-95; Tolstoshev, *Ann Rev Pharmacol Toxicol* 1993, 33: 573-96; Mulligan, *Science* 1993, 260: 926-32; Morgan & Anderson, *Ann Rev Biochem* 1993, 62: 191-217; Trends in Biotechnology 1993, 11(5): 155-215. Methods commonly known in the art of recombinant DNA technology which can also be used for the present invention are described in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, 1993; and Krieger, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY, 1990. Administration methods may be oral, intradermal, subcutaneous, or intravenous injection, and such. A systemic administration or a local administration to the vicinity of the targeted sites is used. The administration can be performed by single administration or boosted by multiple administrations. The polynucleotides of the present invention can be administered to a subject in a therapeutically or pharmaceutically effective dose for inducing immunity (more specifically CTLs) against CDCA1-expressing cancer cells, or in a therapeutically or pharmaceutically effective dose for treating cancer. The dose of a polynucleotide in a suitable carrier or the dose of a polynucleotide in cells transformed with a polynucleotide encoding a peptide of the present invention can be appropriately adjusted according to the disease to be treated, the patient's age and weight, the method of administration and such, and this may be usually 0.001 mg-1000 mg, for example, 0.01 mg-100 mg, for example, 0.1 mg-30 mg, for example, 0.1 mg-10 mg, or for example, 0.5 mg-5 mg. The dosing interval can be once every several days to several months, and for example, the dosing can be done in a once-per-week interval. A skilled artisan can appropriately select a suitable dosage.

X. Methods of Using Peptides, Exosomes, APCs and CTLs

[0230] The peptides and polynucleotides of the present invention can be used to induce APCs and CTLs. CTLs can also be induced using the exosomes and APCs of the present invention. The peptides, polynucleotides, exosomes, and APCs can be used in combination with any other compound (s) as long as their CTL-inducing ability is not inhibited. Therefore, CTLs of the present invention can be induced using a pharmaceutical composition comprising any of the peptides, polynucleotides, APCs and exosomes of the present invention. Further, APCs of the present invention can be induced using a pharmaceutical composition comprising a peptide or polynucleotide of the present invention.

(1) Methods of Inducing APCs

[0231] The present invention provides methods of inducing APCs having CTL-inducing ability, using a peptide(s) or polynucleotide(s) of the present invention.

[0232] The methods of the present invention comprise a step of contacting an APC with a peptide of the present invention *in vitro*, *ex vivo*, or *in vivo*. For example, a method of contacting APCs with the peptide *ex vivo* may comprise the steps below:

[0233] (a) collecting APCs from a subject; and

[0234] (b) contacting the APCs of step (a) with a peptide of the present invention.

[0235] The above-described APCs are not limited to a particular type of cell, and DCs, Langerhans cells, macrophages, B cells, and activated T cells, which are known to present a proteinaceous antigen on their cell surface to be recognized by lymphocytes, can be used. DCs have the most potent CTL-inducing ability among APCs, and thus it is preferable to use DCs. Any peptides of the present invention can be used by themselves or in combination with other peptides of the present invention. Further, peptides of the present invention can be used in combination with other CTL-inducing peptides (for example, other TAA-derived CTL-inducing peptides).

[0236] Meanwhile, when a peptide of the present invention is administered to a subject, APCs are contacted with the peptide *in vivo*, and as a result, APCs having a high CTL-inducing ability are induced in the body of the subject. Therefore, the methods of the present invention may comprise a step of administering a peptide of the present invention to a subject. Similarly, when a polynucleotide of the present invention is administered to a subject in an expressible form, a peptide of the present invention is expressed *in vivo*, the expressed peptide is contacted with APCs *in vivo*, and as a result APCs having a high CTL-inducing ability are induced in the body of the subject. Therefore, the present invention may also comprise a step of administering a polynucleotide of the present invention to a subject.

[0237] In order to induce APCs having CTL-inducing ability, the present invention may comprise a step of introducing a polynucleotide of the present invention into APCs. For example, the method may comprise the steps below:

[0238] (a) collecting APCs from a subject; and

[0239] (b) introducing a polynucleotide encoding a peptide of the present invention into the APCs of step (a).

Step (b) can be performed as described in the above "VI. Antigen-presenting cells (APCs)" section.

[0240] Thus, in one embodiment, the present invention provides a method of inducing APCs having CTL-inducing ability, which comprises the step (a) or (b) below:

[0241] (a) contacting APCs with a peptide of the present invention; or

[0242] (b) introducing a polynucleotide encoding a peptide of the present invention into APCs.

[0243] Furthermore, the present invention provides a method of preparing APCs having CTL-inducing ability, which comprises the step (a) or (b) below:

[0244] (a) contacting APCs with a peptide of the present invention; or

[0245] (b) introducing a polynucleotide encoding a peptide of the present invention into APCs.

[0246] The above-described methods can be performed *in vitro*, *ex vivo*, or *in vivo*, and it is preferable to perform them *in vitro* or *ex vivo*. APCs used in the above-described methods may be derived from a subject scheduled for administration of the induced APCs, or they may be derived from a different subject. When APCs derived from a subject (donor) different from the subject scheduled for administration are used, the subject of administration and the donor must have the identical HLA type. In the methods of the present invention, when a peptide having the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37 or a modified peptide thereof is used as

a peptide of the present invention, the HLA type is preferably HLA-A*01:01 (more preferably HLA-A*01:01) in both the subject of administration and the donor. Alternatively, APCs used in the above-described methods are preferably APCs that express HLA-A*01:01 (more preferably HLA-A*01:01). The APCs can be prepared using known methods from PBMCs after PBMCs are separated from blood collected from a donor by a specific gravity centrifugal method or such.

[0247] In another embodiment, the present invention also provides pharmaceutical compositions that comprise a peptide of the present invention or a polynucleotide encoding the peptide for inducing an APC(s) having CTL-inducing ability.

[0248] Alternatively, the present invention further provides use of a peptide of the present invention or a polynucleotide encoding the peptide in the manufacture of a pharmaceutical composition for inducing an APC(s) having CTL-inducing ability.

[0249] Alternatively, the present invention further provides peptides of the present invention or polynucleotides encoding the peptides for use in the induction of an APC(s) having CTL-inducing ability.

[0250] Alternatively, the present invention further provides methods or processes of manufacturing a pharmaceutical composition for inducing an APC(s), wherein the method or process comprises a step of formulating a peptide of the present invention or a polynucleotide encoding the peptide with a pharmaceutically or physiologically acceptable carrier.

[0251] In another embodiment, the present invention further provides methods or processes of manufacturing a pharmaceutical composition for inducing an APC(s) having CTL-inducing ability, wherein the method or process comprises a step of mixing a peptide of the present invention or a polynucleotide encoding the peptide with a pharmaceutically or physiologically acceptable carrier.

[0252] APCs induced by the methods of the present invention can induce CTLs specific to CDCA1 (i.e., CTLs of the present invention).

(2) Methods of Inducing CTLs

[0253] The present invention also provides methods of inducing CTLs using peptides, polynucleotides, exosomes or APCs of the present invention.

[0254] When a peptide(s), a polynucleotide(s), an exosome(s) or an APC(s) of the present invention is administered to a subject, CTLs are induced in the body of the subject and the strength of the immune response targeting CDCA1-expressing cancer cells is enhanced. Therefore, the methods of the present invention may comprise a step of administering a peptide(s), a polynucleotide(s), an APC(s) or an exosome(s) of the present invention to a subject.

[0255] Alternatively, CTLs can be induced by using them in vitro or ex vivo. For example, the methods of the present invention may include the following steps:

[0256] (a) collecting APCs from a subject;

[0257] (b) contacting the APCs of step (a) with a peptide of the present invention; and

[0258] (c) co-culturing the APCs of step (b) with CD8-positive T cells.

[0259] The induced CTLs may be returned to the subject afterwards.

[0260] The APCs to be co-cultured with the CD8-positive T cells in step (c) above can also be prepared by introducing into APCs a polynucleotide encoding a peptide of the present invention as described above in the “VI. Antigen-presenting cells (APCs)” section. However, the APCs to be used in the methods of the present invention are not limited thereto, and any APCs that present on their surface a complex of an HLA antigen and a peptide of the present invention can be used.

[0261] In the methods of the present invention, instead of such APCs, exosomes that present on their surface a complex of an HLA antigen and a peptide of the present invention can also be used. That is, the methods of the present invention can comprise a step of co-culturing with exosomes that present on their surface a complex of an HLA antigen and a peptide of the present invention. Such exosomes can be prepared by the above-described methods in the “V. Exosomes” section.

[0262] Further, CTLs can also be induced by introducing into a CD8-positive T cell a vector comprising a polynucleotide encoding each subunit of a TCR capable of binding to a peptide of the present invention presented by an HLA antigen on the cell surface. Such transformation can be carried out as described above in the “VIII. T cell receptors (TCRs)” section.

[0263] Accordingly, in one embodiment, the present invention provides methods of inducing CTLs, comprising a step selected from below:

[0264] (a) co-culturing CD8-positive T cells with APCs that present on their surface a complex of an HLA antigen and a peptide of present invention;

[0265] (b) co-culturing CD8-positive T cells with exosomes that present on their surface a complex of an HLA antigen and a peptide of present invention; and

[0266] (c) introducing into CD8-positive T cells, a vector comprising a polynucleotide encoding each subunit of a TCR capable of binding to a peptide of the present invention presented by an HLA antigen on a cell surface.

[0267] The above-described methods can be performed in vitro, ex vivo, or in vivo, and it is preferable to perform them in vitro or ex vivo. APCs or exosomes and CD8-positive T cells used in the above-described methods may be derived from a subject scheduled for administration of the induced CTLs, or they may be derived from a different subject. When APCs or exosomes and CD8-positive T cells derived from a subject (donor) different from the subject scheduled for administration are used, the subject of administration and the donor must have the identical HLA type. For example, when a peptide having the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37 or a modified peptide thereof is used as peptides of the present invention, the HLA type in both the subject of administration and the donor is preferably HLA-A*01:01 (more preferably HLA-A*01:01). Alternatively, APCs or exosomes used in the above-described methods are preferably APCs or exosomes that present on their surface a complex of HLA-A*01:01 (more preferably HLA-A*01:01) and a peptide of the present invention (a peptide having the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37 or a modified peptide thereof). In this case, the induced CTLs show a specific cytotoxic activity against cells that present a complex of HLA-A*01:01 and a

peptide of the present invention (for example, CDCA1-expressing HLA-A01-positive cells).

[0268] In another embodiment, the present invention also provides compositions or pharmaceutical compositions for inducing CTLs, comprising at least one active ingredient selected from below:

[0269] (a) a peptide of the present invention;

[0270] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0271] (c) an APC that presents on its surface a peptide of the present invention; and

[0272] (d) an exosome that presents on its surface a peptide of the present invention.

[0273] In another embodiment, the present invention also provides use of an active ingredient selected from below in the manufacture of compositions or pharmaceutical compositions for inducing CTLs:

[0274] (a) a peptide of the present invention;

[0275] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0276] (c) an APC that presents on its surface a peptide of the present invention; and

[0277] (d) an exosome that presents on its surface a peptide of the present invention.

[0278] Alternatively, the present invention further provides an active ingredient selected from below for use in inducing CTLs:

[0279] (a) a peptide of the present invention;

[0280] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0281] (c) an APC that presents on its surface a peptide of the present invention; and

[0282] (d) an exosome that presents on its surface a peptide of the present invention.

[0283] Alternatively, the present invention further provides a method or process for manufacturing a composition or pharmaceutical composition for inducing CTLs, which is a method or process that comprises a step of formulating an active ingredient selected from below with a pharmaceutically or physiologically acceptable carrier:

[0284] (a) a peptide of the present invention;

[0285] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0286] (c) an APC that presents on its surface a peptide of the present invention; and

[0287] (d) an exosome that presents on its surface a peptide of the present invention.

[0288] In another embodiment, the present invention further provides a method or process for manufacturing a composition or pharmaceutical composition for inducing CTLs, which is a method or process that comprises a step of mixing an active ingredient selected from below with a pharmaceutically or physiologically acceptable carrier:

[0289] (a) a peptide of the present invention;

[0290] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0291] (c) an APC that presents on its surface a peptide of the present invention; and

[0292] (d) an exosome that presents on its surface a peptide of the present invention.

XI. Methods of Inducing an Immune Response

[0293] The present invention further provides methods of inducing an immune response against CDCA1-expressing

cancers. Applicable cancers include bladder cancer, breast cancer, cervical cancer, cholangiocellular cancer, chronic myeloid leukemia (CML), esophagus cancer, gastric cancer, non-small-cell lung cancer, lymphoma, osteosarcoma, prostate cancer, kidney cancer, small-cell lung cancer, head and neck cancer, soft tissue tumor, colon cancer and such, but are not limited thereto. It is preferable that the cancer expresses HLA-A01.

[0294] The present invention further provides methods of inducing an immune response against CDCA1-expressing cancer cells. CDCA1 is recognized to be overexpressed in various types of cancers described above. Thus, when an immune response against CDCA1-expressing cancer cells is induced, proliferation of the cancer cells is inhibited as a result. Accordingly, the present invention further provides methods of inhibiting proliferation of CDCA1-expressing cancer cells. The methods of the present invention are suitable, in particular, for inhibiting proliferation of cancer cells expressing CDCA1 and HLA-A01.

[0295] The methods of the present invention may comprise a step of administering a composition comprising any of the peptides of the present invention or a polynucleotide (s) encoding the peptide(s). The methods of the present invention also contemplate administration of APCs or exosomes presenting any of the peptides of the present invention. The details can be referred to the "IX. Pharmaceutical compositions" section, particularly portions describing regarding use of the pharmaceutical compositions of the present invention as vaccines. In addition, exosomes and APCs that can be used in the methods of the present invention for inducing an immune response are described in detail in "V. Exosomes", "VI. Antigen-presenting cells (APCs)" and in Items (1) and (2) of "X. Methods of using peptides, exosomes, APCs and CTLs" described above.

[0296] In another embodiment, the present invention provides pharmaceutical compositions or vaccines for inducing an immune response against cancers expressing CDCA1 and HLA-A01, wherein the pharmaceutical composition or vaccine comprises an active ingredient selected from below:

[0297] (a) a peptide of the present invention;

[0298] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0299] (c) an APC that presents on its surface a peptide of the present invention;

[0300] (d) an exosome that presents on its surface a peptide of the present invention; and

[0301] (e) a CTL of the present invention.

[0302] Alternatively, the present invention also provides pharmaceutical compositions or vaccines for inducing an immune response against cancer cells expressing CDCA1 and HLA-A01, wherein the pharmaceutical composition or vaccine comprises an active ingredient selected from below:

[0303] (a) a peptide of the present invention;

[0304] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;

[0305] (c) an APC that presents on its surface a peptide of the present invention;

[0306] (d) an exosome that presents on its surface a peptide of the present invention; and

[0307] (e) a CTL of the present invention.

[0308] Alternatively, the present invention further provides pharmaceutical compositions or vaccines for inhibiting proliferation of cancer cells expressing CDCA1 and

HLA-A01, wherein the pharmaceutical composition or vaccine comprises an active ingredient selected from below:

- [0309] (a) a peptide of the present invention;
- [0310] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;
- [0311] (c) an APC that presents on its surface a peptide of the present invention;
- [0312] (d) an exosome that presents on its surface a peptide of the present invention; and
- [0313] (e) a CTL of the present invention.

[0314] In another embodiment, the present invention provides use of an active ingredient selected from below in the manufacture of pharmaceutical compositions or vaccines for inducing an immune response against cancers expressing CDCA1 and HLA-A01:

- [0315] (a) a peptide of the present invention;
- [0316] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;
- [0317] (c) an APC that presents on its surface a peptide of the present invention;
- [0318] (d) an exosome that presents on its surface a peptide of the present invention; and
- [0319] (e) a CTL of the present invention.

[0320] Alternatively, the present invention also provides use of an active ingredient selected from below in the manufacture of pharmaceutical compositions or vaccines for inducing an immune response against cancer cells expressing CDCA1 and HLA-A01:

- [0321] (a) a peptide of the present invention;
- [0322] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;
- [0323] (c) an APC that presents on its surface a peptide of the present invention;
- [0324] (d) an exosome that presents on its surface a peptide of the present invention; and
- [0325] (e) a CTL of the present invention.

[0326] Alternatively, the present invention further provides use of an active ingredient selected from below in the manufacture of pharmaceutical compositions or vaccines for inhibiting proliferation of cancer cells expressing CDCA1 and HLA-A01:

- [0327] (a) a peptide of the present invention;
- [0328] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;
- [0329] (c) an APC that presents on its surface a peptide of the present invention;
- [0330] (d) an exosome that presents on its surface a peptide of the present invention; and
- [0331] (e) a CTL of the present invention.

[0332] The present invention further provides methods or processes for manufacturing pharmaceutical compositions that induce an immune response against cancers expressing CDCA1 and HLA-A01, which is a method that may comprise a step of mixing or formulating a peptide of the present invention with a pharmaceutically acceptable carrier.

[0333] Alternatively, the present invention provides methods for inhibiting proliferation of cancer cells expressing CDCA1 and HLA-A01 in diseased sites of diseases mediated by angiogenesis or methods of inducing an immune response against cancers, which comprises a step of administering to a subject vaccines or pharmaceutical compositions comprising an active ingredient selected from below:

- [0334] (a) a peptide of the present invention;
- [0335] (b) a polynucleotide encoding a peptide of the present invention in an expressible form;
- [0336] (c) an APC that presents a peptide of the present invention on its surface;
- [0337] (d) an exosome that presents a peptide of the present invention on its surface; and
- [0338] (e) a CTL of the present invention.

[0339] In the context of the present invention, cancers expressing CDCA1 and HLA-A01 can be treated by administering a peptide, a polynucleotide, an APC, an exosome and/or a CTL of the present invention. Alternatively, an immune response against CDCA1-expressing cancers can be induced by administering a peptide, a polynucleotide, an APC, an exosome and/or a CTL of the present invention. Examples of such cancers include bladder cancer, breast cancer, cervical cancer, cholangiocellular cancer, chronic myeloid leukemia (CML), esophagus cancer, gastric cancer, non-small-cell lung cancer, lymphoma, osteosarcoma, prostate cancer, kidney cancer, small-cell lung cancer, head and neck cancer, soft tissue tumor, colon cancer and such, but are not limited thereto. Further, an immune response against cancer cells expressing CDCA1 and HLA-A01 can be induced by administering a peptide, a polynucleotide, an APC, an exosome and/or a CTL of the present invention. Therefore, before administering a vaccine or pharmaceutical composition comprising an active ingredient described above, it is preferable to confirm whether the level of CDCA1 expression at a diseased site in the subject to be treated is augmented or not. Similarly, it is also desirable to confirm that the subject to be treated is HLA-A01-positive. That is, in a preferred embodiment, the present invention can select a subject who is HLA-A01-positive and who has cancer expressing CDCA1 and administer the vaccine or the pharmaceutical composition of the present invention to the selected subject.

[0340] Thus, in one embodiment, the present invention provides a method of treating a cancer expressing CDCA1 and HLA-A01 in a patient in need of the cancer treatment, wherein the method comprises the steps below:

- [0341] (i) measuring the level of CDCA1 expression in a biological sample collected from the diseased site of an HLA-A01-positive subject with cancer;
- [0342] (ii) identifying a subject with CDCA1-expressing cancer based on the CDCA1 expression level measured in (i); and
- [0343] (iii) administering to the subject with CDCA1-overexpressing cancer as compared to normal control at least one ingredient selected from the group consisting of (a) to (e) above.

[0344] Alternatively, the present invention further provides vaccines or pharmaceutical compositions comprising at least one active ingredient selected from the group consisting of (a) to (e) above for administration to an HLA-A01-positive subject with CDCA1-expressing cancer. The present invention further provides a method of identifying or selecting a subject to be treated with at least one active ingredient selected from the group consisting of (a) to (e) above, wherein the method comprises the steps below:

- [0345] (i) measuring the level of CDCA1 expression in a biological sample collected from the diseased site of an HLA-A01-positive subject with cancer;
- [0346] (ii) identifying a subject with CDCA1-expressing cancer based on the CDCA1 expression level measured in (i); and

[0347] (iii) identifying or selecting the subject identified in (ii) as a subject who may be treated with at least one active ingredient selected from the group consisting of (a) to (e) above.

[0348] Biological samples collected from a subject for measuring the CDCA1 expression level in the above-described methods are not particularly limited, and for example, tissue samples containing cancer cells collected by biopsy or such can be preferably used. The CDCA1 expression level in a biological sample can be measured by known methods, and for example, methods that detect transcription products of the CDCA1 gene by probes or PCR methods (for example, cDNA microarray method, Northern blot method, RT-PCR method or such), methods that detect translation products of the CDCA1 gene by antibodies or such (for example, Western blot method, immunostaining method or such), and such can be used. Further, biological samples may be blood samples, and in this case, the blood level of an antibody against CDCA1 is measured, and the CDCA1 expression level at a diseased site may be assessed based on the blood level. The blood level of an antibody against CDCA1 can be measured using known methods, and for example, enzyme immunoassay (EIA), enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and such using the CDCA1 protein or a peptide of the present invention as an antigen can be used. Alternatively, the CDCA1 expression level at a diseased site may be assessed by detecting CTLs specific to a peptide of the present invention. The CTL levels specific to a peptide of the present invention can be measured, for example, by separating PBMCs from the blood collected from a subject and measuring the cytotoxic activity against target cells pulsed with the peptide of the present invention. The cytotoxic activity can be measured, for example, by the amount of interferon-gamma release. Furthermore, complexes of the peptides of the present invention and HLA described below can also be used for measuring the CTL levels. Whether a subject's cancer expresses CDCA1 or not may be determined by comparing with the measurement results in a biological material of the same type collected from a subject without cancer. That is, if the level of a substance to be measured in a biological sample collected from a subject having cancer is elevated relative to the level in a biological material of the same type collected from another subject without cancer (normal control level), it can be determined that the cancer of the subject having cancer expresses CDCA1.

[0349] In a preferred embodiment, it is preferable to confirm the HLA type of a subject before administering at least one active ingredient selected from the group consisting of (a) to (e) above. For example, it is preferable to select an HLA-A01-positive subject as a subject to be administered with an active ingredient which is related to a peptide having the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37. The HLA of a subject can be confirmed by immunologically detecting an HLA on the surface of cells collected from the subject with an HLA-A01-specific antibody. Alternatively, the HLA of a subject can be determined by analyzing the genetic information of genomic DNA or mRNA obtained from cells collected from the subject. If a subject is HLA-A01-positive, then effective treatment can be expected in the present invention. In other words, a subject having homozygous or heterozygous HLA-A01 can be a target of administration.

[0350] The present invention further provides complexes of a peptide of the present invention and HLA. The complexes of the present invention described above may be monomers or multimers. When a complex of the present invention is a multimer, the number of polymerization is not particularly limited, and it can be a multimer of any number of polymerization. Examples include a tetramer, pentamer, hexamer and such, but are not limited thereto. The multimers of the present invention also encompass dextramers (WO2002/072631) and streptamers (Knabel M et al., Nat Med. 2002; 8(6): 631-7.). Complexes of a peptide of the present invention and HLA can be prepared according to known methods (for example, Altman J D et al., Science. 1996, 274(5284): 94-6; WO2002/072631; WO2009/003492; Knabel M et al., Nat Med. 2002; 8(6): 631-7, and such). The complexes of the present invention, for example, can be used in the quantification of CTLs specific to a peptide of the present invention. For example, a blood sample is collected from a subject administered with a pharmaceutical composition of the present invention, and CD4-negative cells are adjusted after separation of PBMCs and contacted with a fluorescent dye-conjugated complex of the present invention. Then, the percentage of CTLs specific to a peptide of the present invention can be measured by flow cytometry analysis. For example, immune response-inducing effects by a pharmaceutical composition of the present invention can be monitored by measuring the specific CTLs against a peptide of the present invention before, during and/or after administration of the pharmaceutical composition of the present invention.

XII. Antibodies

[0351] The present invention further provides antibodies that bind to the peptide of the present invention. Preferable antibodies bind specifically to a peptide of the present invention, but do not bind (or weakly bind) to one that is not the peptide of the present invention. The binding specificity of an antibody can be confirmed by inhibition assay. That is, if the binding between an antibody to be analyzed and a full-length CDCA1 polypeptide is inhibited in the presence of a peptide of the present invention, this antibody is shown to specifically bind to the peptide of the present invention. Antibodies against peptides of the present invention can be used in assays of disease diagnosis and prognosis, as well as subject selection for administration of the pharmaceutical compositions of the present invention and monitoring of the pharmaceutical compositions of the present invention.

[0352] The present invention also provides various immunological assays for detecting and/or quantifying peptides of the present invention or fragments thereof. Such immunological assays include radioimmunoassay, immunochromatography, enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunofluorescence assay (ELIFA) and such, without being limited thereto, and are performed within the scope of the various immunological assay formats well known in the art.

[0353] The antibodies of the present invention can be used in immunological imaging methods that can detect CDCA1-expressing diseases, and examples thereof include radioactive scintigraphic imaging using a labelled antibody of the present invention, without being limited thereto. Such assay methods are used clinically in the detection, monitoring, and prognosis of CDCA1-expressing cancers; and examples of such cancer include bladder cancer, breast cancer, cervical

cancer, cholangiocellular cancer, chronic myeloid leukemia (CML), esophagus cancer, gastric cancer, non-small-cell lung cancer, lymphoma, osteosarcoma, prostate cancer, kidney cancer, small-cell lung cancer, head and neck cancer, soft tissue tumor, colon cancer and such, without being limited thereto.

[0354] The antibodies of the present invention can be used in any arbitrary form such as monoclonal antibodies or polyclonal antibodies, and may further include anti-sera obtained by immunizing an animal such as a rabbit with a peptide of the present invention, all classes of polyclonal antibodies and monoclonal antibodies, human antibodies, as well as chimeric antibodies and humanized antibodies generated through gene recombination.

[0355] The peptide of the present invention or a fragment thereof used as an antigen for obtaining antibodies can be obtained by chemical synthesis or genetic engineering techniques based on the amino acid sequences disclosed herein.

[0356] The peptide used as an immunizing antigen may be a peptide of the present invention or a fragment of a peptide of the present invention. Further, the peptide may be bound to or conjugated with a carrier for increasing immunogenicity. Keyhole limpet hemocyanin (KLH) is well-known as a cancer. Methods for binding KLH to a peptide are also well known in the art.

[0357] Any mammal can be immunized with an antigen described above, and it is preferable to consider the compatibility with the parent cell used in cell fusion when generating a monoclonal antibody. Generally, animals of the order Rodentia, Lagomorpha or Primate can be used. Animals of the order Rodentia include, for example, mice, rats and hamsters. Animals of the order Lagomorpha include, for example, rabbits. Animals of the order Primate include, for example, Catarrhini monkeys (old world monkeys) such as cynomolgus monkey (*Macaca fascicularis*), rhesus monkeys, hamadryas, and chimpanzee.

[0358] Methods of immunizing animals with an antigen are known in the art. Intraperitoneal injection and subcutaneous injection of an antigen are standard methods for immunizing mammals. More specifically, an antigen is diluted and suspended in an appropriate amount of phosphate buffered saline (PBS), physiological saline, or such. As needed, an antigen suspension solution can be administered to mammals after being mixed with an appropriate amount of a standard adjuvant such as Freund's complete adjuvant and emulsified. Then, it is preferable to administer the antigen mixed with an appropriate amount of a Freund's incomplete adjuvant several times every 4 to 21 days. A suitable carrier may be used for immunization. After the above immunization, the serum can be examined by standard method with respect to increase in the quantity of the desired antibody.

[0359] Polyclonal antibodies against a peptide of the present invention can be prepared by collecting blood from mammals that have been confirmed with an increase in the serum level of the desired antibody after immunization, and separating the serum from blood by any conventional method. A polyclonal antibody may be a polyclonal antibody-containing serum, or a polyclonal antibody-containing fraction may be isolated from the serum. Immunoglobulin G or M can be prepared from fractions that recognize only a peptide of the present invention by, for example, using an affinity column conjugated with the peptide of the present

invention, and then further purifying the fractions using a protein A or protein G column

[0360] In order to prepare monoclonal antibodies, upon confirming an increase in the serum level of the desired antibody after immunization, immune cells are collected from the mammals and subjected to cell fusion. Immune cells used for cell fusion may be preferably obtained from the spleen. For the other parent cells fused with the above immune cells, for example, a mammalian myeloma cell, preferably a myeloma cell that has acquired a property for drug selection of fusion cells can be used.

[0361] The above immune cells can be fused with myeloma cells by following known methods, for example, the method of Milstein et al. (Galfre and Milstein, *Methods Enzymol* 73: 1981, 3-46).

[0362] Hybridomas obtained by cell fusion can be selected by culturing them in a standard selection medium such as the HAT medium (a medium containing hypoxanthine, aminopterin and thymidine). Cell culturing is typically continued in the HAT medium for a sufficient period of time (for example, several days to several weeks) to allow death of all other cells (non-fused cells) besides the desired hybridomas. Then, hybridoma cells producing the desired antibody can be screened and cloned by performing a standard limiting dilution.

[0363] In addition to the above methods of immunizing a non-human animal with an antigen for hybridoma preparation, human lymphocytes such as EB virus-infected lymphocytes can be immunized in vitro with a peptide, cells expressing the peptide, or lysates thereof. Then, the immunized lymphocytes can be fused with immortalized human-derived myeloma cells such as U266 to obtain hybridomas producing a desired human antibody capable of binding to the peptide (JPS63-17688).

[0364] Next, the obtained hybridoma is transplanted into the abdominal cavity of a mouse, and the ascites is extracted. The obtained monoclonal antibody can be purified by, for example, ammonium sulfate precipitation, protein A or protein G column, DEAE ion-exchange chromatography, or affinity column conjugated with the peptide of the present invention.

[0365] Alternatively, antibody-producing immune cells such as the immunized lymphocytes can be immortalized by a cancer gene and used for the preparation of monoclonal antibodies.

[0366] The monoclonal antibodies obtained as such can also be prepared by recombination using genetic engineering techniques (see, e.g., Borrebaeck and Larrick, *Therapeutic Monoclonal Antibodies* published in United Kingdom by MacMillan Publishers LTD (1990)). For example, an antibody-encoding DNA can be cloned from immune cells such as antibody-producing hybridoma or immunized lymphocytes and inserted into a suitable vector, and then this is introduced into host cells to prepare a recombinant antibody. The present invention also provides recombinant antibodies prepared as described above.

[0367] Further, the antibodies of the present invention may be antibody fragments or modified antibodies, as long as they bind to the peptides of the present invention. For example, the antibody fragments may be Fab, F(ab')₂, Fv, or a single chain Fv (scFv) in which Fv fragments derived from an H chain and an L chain are linked with a suitable linker (Huston et al., *Proc Natl Acad Sci USA* 1988, 85: 5879-83). More specifically, antibody fragments may be generated by

treating an antibody with an enzyme such as papain or pepsin. Alternatively, a gene encoding an antibody fragment may be constructed, inserted into an expression vector, and expressed in an appropriate host cell (see, e.g., Co et al., *J Immunol* 1994, 152: 2968-76; Better and Horwitz,

[0368] *Methods Enzymol* 1989, 178: 476-96; Pluckthun and Skerra, *Methods Enzymol* 1989, 178: 497-515; Lamoyi, *Methods Enzymol* 1986, 121: 652-63; Rousseaux et al., *Methods Enzymol* 1986, 121: 663-9; Bird and Walker, *Trends Biotechnol* 1991, 9: 132-7).

[0369] Antibodies may be modified by conjugation with various molecules such as polyethyleneglycol (PEG). The present invention provides such modified antibodies. Modified antibodies can be obtained by chemically modifying the antibodies. These modification methods are conventional in the art.

[0370] Alternatively, the antibodies of the present invention can be obtained as chimeric antibodies of a non-human antibody-derived variable region and a human antibody-derived constant region, or as humanized antibodies comprising a non-human antibody-derived complementarity determining region (CDR) and a human antibody-derived framework region (FR) and constant region. Such antibodies can be prepared according to known techniques. Humanization can be carried out by substituting a human antibody sequence(s) with a corresponding non-human antibody CDR sequence(s) (see, e.g., Verhoeven et al., *Science* 1988, 239: 1534-6). Thus, such humanized antibodies are chimeric antibodies in which the substantially less than an intact human variable domain has been substituted with a corresponding sequence from a non-human species.

[0371] Intact human antibodies comprising a human variable region in addition to the human framework and constant regions can also be used. Such antibodies can be generated using various techniques known in the art. For example, in vitro methods include use of recombinant libraries of human antibody fragments presented on bacteriophages (for example, Hoogenboom & Winter, *J. Mol. Biol.* 1991, 227: 381). Similarly, human antibodies can also be generated by introducing human immunoglobulin gene loci into transgenic animals, for example, mice, in which the endogenous immunoglobulin genes have been partially or completely inactivated. This approach is described in, for example, U.S. Pat. Nos. 6,150,584, 5,545,807, 5,545,806, 5,569,825, 5,625,126, 5,633,425 and 5,661,016.

[0372] Antibodies obtained as described above may be purified to homogeneity. For example, antibody separation and purification can be performed according to separation methods and purification methods used for general proteins. For example, an antibody can be separated and isolated by appropriately selecting and combining use of column chromatographies such as affinity chromatography, filter, ultra-filtration, salting-out, dialysis, SDS-polyacrylamide gel electrophoresis and isoelectric focusing electrophoresis (*Antibodies: A Laboratory Manual*. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)), but are not limited thereto. Protein A column and protein G column can be used as the affinity column. Exemplary protein A columns to be used include, for example, Hyper D, POROS and Sepharose F. F. (Pharmacia).

[0373] Besides affinity chromatography, exemplary chromatography includes, for example, ion-exchange chromatography, hydrophobic chromatography, gel filtration, reversed-phase chromatography, adsorption chromatogra-

phy and such (*Strategies for Protein Purification and Characterization: A Laboratory Course Manual*. Ed Daniel R. Marshak et al., Cold Spring Harbor Laboratory Press (1996)). The chromatography procedures can be carried out by liquid-phase chromatography such as HPLC and FPLC.

[0374] The antigen-binding activity of an antibody of the present invention can be measured, for example, by using absorbance measurement, enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA), and/or immunofluorescence (IF). In the case of ELISA, an antibody of the present invention is immobilized onto a plate, a peptide of the present invention is applied to the plate, and then a sample containing the desired antibody, such as culture supernatant of antibody-producing cells or purified antibodies, is applied. Next, a secondary antibody that recognizes the primary antibody and is labelled with an enzyme such as alkaline phosphatase is applied and the plate is incubated. Then, after washing, an enzyme substrate such as p-nitrophenyl phosphate is applied to the plate, and the antigen-binding activity of the sample is evaluated by measuring absorbance. To assess the binding activity of an antibody, peptide fragments such as C-terminal or N-terminal fragments may be used as an antigen. BIAcore (Pharmacia) may be used to evaluate the activity of an antibody of the present invention.

[0375] It is possible to detect or measure a peptide of the present invention using the above methods, by exposing an antibody of the present invention to a sample assumed to contain the peptide of the present invention, and detecting or measuring an immune complex formed between the antibody and the peptide.

[0376] For example, an antibody of the present invention can be used to detect a peptide of the present invention present in the blood sample (for example, serum sample) of a subject. Alternatively, an antibody of the present invention present in the blood sample (for example, serum sample) of a subject can also be detected using a peptide of the present invention. The result of measuring a peptide of the present invention or an antibody of the present invention in the blood sample of a subject can be utilized to the subject selection for administration of the pharmaceutical compositions of the present invention or monitoring of the efficacy of the pharmaceutical compositions of the present invention.

XIII. Vectors and Host Cells

[0377] The present invention provides vectors comprising a polynucleotide encoding a peptide of the present invention and host cells introduced with the vectors. A vector of the present invention may be used to keep a polynucleotide of the present invention in a host cell, to express a peptide of the present invention in a host cell, or to administer a polynucleotide of the present invention for gene therapy.

[0378] When *E. coli* is a host cell and a vector is amplified and produced in a large amount in *E. coli* (for example, JM109, DH5-alpha, HB101 or XL1-Blue), the vector needs to have a "replication origin" for amplification in *E. coli* and a marker gene for selection of transformed *E. coli* (for example, a drug resistance gene selected by a drug such as ampicillin, tetracycline, kanamycin, chloramphenicol). For example, the M13-series vectors, pUC-series vectors, pBR322, pBluescript, pCR-Script and such can be used. In addition, pGEM-T, pDIRECT and pT7 can be used for cloning as well as the above vectors. When a vector is used in the production of a peptide of the present invention, an

expression vector can be used. For example, an expression vector for expression in *E. coli* needs to have the above features for amplification in *E. coli*. When *E. coli* such as JM109, DHS-alpha, HB101 or XL1-Blue are used as a host cell, the vector needs to have a promoter, for example, lacZ promoter (Ward et al., Nature 1989, 341: 544-6; FASEB J. 1989, 6: 2422-7), araB promoter (Better et al., Science 1988, 240: 1041-3), T7 promoter or the like, that can efficiently express the desired gene in *E. coli*. In that respect, pGEX-5X-1 (Pharmacia), "QIAexpress system" (Qiagen), pEGFP and pET (in this case, the host is preferably BL21 which expresses T7 RNA polymerase), for example, can be used instead of the above vectors. Additionally, the vector may contain a signal sequence for peptide secretion. An exemplary signal sequence that directs the peptide to be secreted to the periplasm of the *E. coli* is the pelB signal sequence (Lei et al., J Bacteriol. 1987, 169: 4379). Means for introducing the vectors into the target host cells include, for example, the calcium chloride method and the electroporation method.

[0379] In addition to *E. coli*, for example, expression vectors derived from mammals (for example, pcDNA3 (Invitrogen) and pEGF-BOS (Nucleic Acids Res 1990, 18(17): 5322), pEF, pCDM8), expression vectors derived from insect cells (for example, "Bac-to-BAC baculovirus expression system" (GIBCO BRL), pBacPAK8), expression vectors derived from plants (e.g., pMH1, pMH2), expression vectors derived from animal viruses (e.g., pHSV, pMV, pAdexLcw), expression vectors derived from retroviruses (e.g., pZIpneo), expression vectors derived from yeast (e.g., "Pichia Expression Kit" (Invitrogen), pNV11, SP-Q01) and expression vectors derived from *Bacillus subtilis* (e.g., pPL608, pKTH50) can be used for producing the polypeptide of the present invention.

[0380] In order to express the vector in animal cells such as CHO, COS or NIH3T3 cells, the vector needs to carry a promoter necessary for expression in such cells, for example, the SV40 promoter (Mulligan et al., Nature 1979, 277: 108), the MMLV-LTR promoter, the EF1-alpha promoter (Mizushima et al., Nucleic Acids Res. 1990, 18: 5322), the CMV promoter and the like, and preferably a marker gene for selecting transformants (for example, a drug resistance gene selected by a drug (e.g., neomycin, G418)). Examples of known vectors with these characteristics include, for example, pMAM, pDR2, pBK-RSV, pBK-CMV, pOPRSV and pOP13.

[0381] The embodiments of the present invention are exemplified below based on the above explanation; however, the present invention is not limited to these embodiments.

[0382] [1] A peptide of less than 15 amino acids having cytotoxic T cell (CTL)-inducing ability, which comprises the amino acid sequence selected from the group of:

[0383] (a) the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37; and

[0384] (b) the amino acid sequence in which one, two, three or several amino acids are substituted, deleted, inserted and/or added to the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37.

[0385] [2] The peptide of [1], comprising the amino acid sequence in which one or more (i.e., one, two or three) substitution(s) selected from (a) to (c) below is introduced

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

[0386] (a) the second amino acid from the N terminus is substituted with an amino acid selected from the group consisting of threonine and serine;

[0387] (b) the third amino acid from the N terminus is substituted with an amino acid selected from the group consisting of aspartic acid and glutamic acid; and

[0388] (c) the C-terminal amino acid is substituted with tyrosine.

[0389] [3] The peptide of [1], which consists of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37.

[0390] [4] A polynucleotide, which encodes the peptide of any one of [1] to [3].

[0391] [5] A composition comprising a pharmaceutically acceptable carrier and at least one ingredient selected from the group consisting of (a) to (e) below:

[0392] (a) one or more types of peptides of any one of [1] to [3];

[0393] (b) one or more types of polynucleotides encoding the peptide(s) of any one of [1] to [3] in an expressible form;

[0394] (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen;

[0395] (d) an exosome that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen; and

[0397] (e) a CTL that targets the peptide of any one of [1] to [3].

[0398] [6] The composition of [5], which is a composition for inducing a CTL(s), wherein the ingredient is at least one ingredient selected from the group consisting of (a) to (d) below:

[0399] (a) one or more types of peptides of any one of [1] to [3];

[0400] (b) one or more types of polynucleotides encoding the peptide(s) of any one of [1] to [3] in an expressible form;

[0401] (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen; and

[0402] (d) an exosome that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen.

[0403] [7] The composition of [5], which is a pharmaceutical composition.

[0405] [8] The composition of [7], which is a pharmaceutical composition for one or more uses selected from the group consisting of (i) cancer treatment, (ii) cancer prevention (prophylaxis) and (iii) prevention (prophylaxis) of postoperative cancer recurrence.

[0406] [9] The composition of [7], which is for inducing an immune response against cancer.

[0407] [10] The composition of [8] or [9], wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangiocellular cancer, chronic myeloid leukemia (CML), esophagus cancer, gastric cancer, non-small-cell lung cancer, lymphoma, osteosarcoma, prostate cancer, kidney cancer, small-cell lung cancer, head and neck cancer, soft tissue tumor and colon cancer.

- [0408] [11] The composition of any one of [5] to [10], which is formulated for administration to a subject positive for HLA-A01.
- [0409] [12] A method of inducing an APC(s) having CTL-inducing ability, which comprises a step selected from the group consisting of:
- [0410] (a) contacting an APC(s) with the peptide of any one of [1] to [3] in vitro, ex vivo or in vivo; and
- [0411] (b) introducing a polynucleotide encoding the peptide of any one of [1] to [3] into an APC(s).
- [0412] [13] A method of inducing a CTL(s), which comprises a step selected from the group consisting of (a) to (c) below:
- [0413] (a) co-culturing a CD8-positive T cell(s) with an APC(s) that presents on its surface a complex of an HLA antigen and the peptide of any one of [1] to [3];
- [0414] (b) co-culturing a CDR-positive T cell(s) with an exosome(s) that presents on its surface a complex of an HLA antigen and the peptide of any one of [1] to [3]; and
- [0415] (c) introducing into a CD8-positive T cell(s) a polynucleotide encoding each subunit of a T cell receptor (TCR) capable of binding to the peptide of any one of [1] to [3] presented by an HLA antigen on a cell surface.
- [0416] [14] An APC that presents on its surface a complex of an HLA antigen and the peptide of any one of [1] to [3].
- [0417] [15] The APC of [14], which is induced by the method of [12].
- [0418] [16] A CTL that targets the peptide of any one of [1] to [3].
- [0419] [17] The CTL of [16], which is induced by the method of [13].
- [0420] [18] A method of inducing an immune response against cancer, which comprises administering to a subject at least one ingredient selected from the group consisting of (a) to (e) below:
- [0421] (a) one or more types of peptides of any one of [1] to [3];
- [0422] (b) one or more types of polynucleotides encoding the peptide(s) of any one of [1] to [3] in an expressible form;
- [0423] (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen;
- [0424] (d) an exosome that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen; and
- [0425] (e) a CTL that targets the peptide of any one of [1] to [3].
- [0426] [19] A method of treating and/or preventing cancer, and/or preventing postoperative recurrence thereof, which comprises administering to a subject at least one ingredient selected from the group consisting of (a) to (e) below:
- [0427] (a) one or more types of peptides of any one of [1] to [3];
- [0428] (b) one or more types of polynucleotides encoding the peptide(s) of any one of [1] to [3] in an expressible form;
- [0429] (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen;
- [0430] (d) an exosome that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen; and
- [0431] (e) a CTL that targets the peptide of any one of [1] to [3].
- [0432] [3] and an HLA antigen; and
- [0433] (e) a CTL that targets the peptide of any one of [1] to [3].
- [0434] [20] An antibody that binds to the peptide of any one of [1] to [3].
- [0435] [21] A method of screening for a peptide having CTL-inducing ability, which comprises the steps of:
- [0436] (a) generating candidate sequences consisting of an amino acid sequence in which one, two or several amino acid residues are substituted, deleted, inserted and/or added to an original amino acid sequence consisting of the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37;
- [0437] (b) selecting from among the candidate sequences generated in (a), a candidate sequence that does not have significant homology (sequence identity) with any known human gene product other than CDCA1;
- [0438] (c) contacting an APC(s) with a peptide consisting of the candidate sequence selected in (b);
- [0439] (d) contacting the APC(s) of (c) with a CD8-positive T cell(s); and
- [0440] (e) selecting a peptide having an equal to or higher CTL-inducing ability than that of a peptide consisting of the original amino acid sequence.
- [0441] [22] Use of at least one active ingredient selected from the group consisting of (a) to (e) below in the manufacture of a composition for inducing an immune response against cancer:
- [0442] (a) one or more types of peptides of any one of [1] to [3];
- [0443] (b) one or more types of polynucleotides encoding the peptide(s) of any one of [1] to [3] in an expressible form;
- [0444] (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen;
- [0445] (d) an exosome that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen; and
- [0446] (e) a CTL that targets the peptide of any one of [1] to [3].
- [0447] [23] Use of at least one ingredient selected from the group consisting of (a) to (e) below in the manufacture of a pharmaceutical composition for treating and/or preventing cancer, and/or preventing postoperative recurrence thereof:
- [0448] (a) one or more types of peptides of any one of [1] to [3];
- [0449] (b) one or more types of polynucleotides encoding the peptide(s) of any one of [1] to [3] in an expressible form;
- [0450] (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen;
- [0451] (d) an exosome that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen; and
- [0452] (e) a CTL that targets the peptide of any one of [1] to [3].
- [0453] [24] Use of at least one ingredient selected from the group consisting of (a) to (e) below in the manufacture of a pharmaceutical composition for treating and/or preventing cancer, and/or preventing postoperative recurrence thereof:
- [0454] (a) one or more types of peptides of any one of [1] to [3];
- [0455] (b) one or more types of polynucleotides encoding the peptide(s) of any one of [1] to [3] in an expressible form;
- [0456] (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen;
- [0457] (d) an exosome that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen; and
- [0458] (e) a CTL that targets the peptide of any one of [1] to [3].

[0454] [24] Use of at least one ingredient selected from the group consisting of (a) to (e) below for inducing an immune response against cancer:

[0455] (a) one or more types of peptides of any one of [1] to [3];

[0456] (b) one or more types of polynucleotides encoding the peptide(s) of any one of [1] to [3] in an expressible form;

[0457] (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen;

[0458] (d) an exosome that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen; and

[0459] (e) a CTL that targets the peptide of any one of [1] to [3].

[0460] [25] Use of at least one ingredient selected from the group consisting of (a) to (e) below for treating and/or preventing cancer and/or preventing postoperative recurrence thereof:

[0461] (a) one or more types of peptides of any one of [1] to [3];

[0462] (b) one or more types of polynucleotides encoding the peptide(s) of any one of [1] to [3] in an expressible form;

[0463] (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen;

[0464] (d) an exosome that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen; and

[0465] (e) a CTL that targets the peptide of any one of [1] to [4].

[0466] [26] A method of inducing cytotoxic activity against a CDCA1-expressing cell(s), which comprises a step of administering to a subject at least one ingredient selected from the group consisting of (a) to (e) below:

[0467] (a) one or more types of peptides of any one of [1] to [3];

[0468] (b) one or more types of polynucleotides encoding the peptide(s) of any one of [1] to [3] in an expressible form;

[0469] (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen;

[0470] (d) an exosome that presents on its cell surface a complex of the peptide of any one of [1] to [3] and an HLA antigen; and

[0471] (e) a CTL that targets the peptide of any one of [1] to [3].

[0472] [27] A freeze-dried formulation comprising one or more types of peptides of any one of [1] to [3].

[0473] [28] A pharmaceutical composition, which is prepared by a method that comprises dissolving one or more types of peptides of any one of [1] to [3] in a water-soluble carrier, and performing filtration sterilization.

[0474] [29] A filtration-sterilized aqueous solution, which is an aqueous solution that comprises one or more types of peptides of any one of [1] to [3] and a water-soluble carrier.

[0475] [30] An emulsion comprising one or more types of peptides of any one of [1] to [3], a water-soluble carrier and an oil adjuvant.

[0476] [31] A kit comprising a container that houses the composition of any one of [5] to [11] and a container that stores an adjuvant.

[0477] [32] A kit comprising a container that stores a freeze-dried formulation comprising the peptide of any one of [1] to [3], a container that stores an adjuvant, and a container that stores a re-dissolving solution for the freeze-dried formulation.

[0478] Preferably, the kit of the present invention further comprises an HLA-A*01 detection reagent additionally.

[0479] The present invention is explained herein in detail with reference to its specific embodiments. However, it should be understood that the above explanation is in fact an illustrative and explanatory explanation, and is intended to explain the present invention and preferred embodiments thereof. Through routine experimentation, one skilled in the art will readily recognize that various changes and modifications can be made therein without departing from the spirit and scope of the present invention. Thus, the present invention is not confined to the above explanation, but is intended to be defined by the appended claims and equivalents thereto.

[0480] Hereinbelow, the present invention is described in more detail with reference to the Examples. Nevertheless, while the following materials, method and Examples may serve to assist one of ordinary skill in making and using certain embodiments of the present invention, there are only intended to illustrate aspects of the present invention and thus in no way to limit the scope of the present invention. One of ordinary skill in the art can use methods and materials similar or equivalent to those described herein in the practice or testing of the present invention.

Examples

Materials and Methods

Cell Lines

[0481] C1R, an HLA-A- and HLA-B-negative human B lymphoblastoid cell line, and COS7, an African green monkey kidney cell line, were purchased from ATCC.

Generation of Target Cells with Constitutive HLA-A*01:01 Expression

[0482] C1R cells (C1R-A01) that constitutively express HLA-A*01:01 was used as cells for stimulating CTLs. A cDNA encoding the HLA-A*01:01 gene was amplified by PCR and introduced into an expression vector. C1R cells incorporated with HLA-A*01:01 gene expression vector were cultured for two weeks under drug selection with a medium containing G418 (Invitrogen). After diluting G418-resistant C1R cell suspension, the cells were seeded into a 96-well plate, and subjected to selective culture for another 30 days with a medium containing G418. The HLA-A*01:01 expression in C1R cells was verified by flow cytometric analysis.

Selection of CDCA1-Derived Peptides

[0483] CDCA1-derived 8 mer, 9 mer and 10 mer peptides that are expected to bind to HLA-A*01:01 were determined using the binding prediction server "NetMHC 3.2" (www.cbs.dtu.dk/services/NetMHC-3.2) (Buus et al., Tissue Antigens. 2003, 62(5): 378-84; Nielsen et al., Protein Sci. 2003, 12(5): 1007-17; Lundegaard C et al., Bioinformatics. 2008, 24(11): 1397-98), "BIMAS" (<http://www.bimas.cit.nih.gov/>)

molbio/hla_bind/) (Parker K C et al., J Immunol 1994, 152(1): 163-75) and “SYFPEITHI”(http://www.syfpeithi.de/bin/MHCServer.d11/EpitopePrediction.htm) (Ram-mensee H G et al., Immunogenetics 1999, 50(3-4): 213-9).

Peptide Synthesis

[0484] The peptides were chemically synthesized by American Peptide Company (Sunnyvale, Calif.) according to a standard solid-phase synthesis method, and purified by reversed phase high-performance liquid chromatography (HPLC). The quality (purity of 90% or higher) of the peptides was guaranteed by HPLC and mass spectrometry. The peptides were dissolved in dimethylsulfoxide (final concentration: 20 mg/ml) and stored at -80°C .

In Vitro CTL Induction

[0485] Monocyte-derived dendritic cells (DCs) were used as the antigen-presenting cell to induce a cytotoxic T lymphocyte (CTL) specific to peptides presented on human leukocyte antigens (HLAs). DCs were prepared in vitro as already reported in the literature (Nakahara S et al., Cancer Res 2003, 63(14): 4112-8). Specifically, peripheral-blood mononuclear cells

[0486] (PBMCs) collected from healthy volunteers (HLA-A*01:01-positive) with the Ficoll-Paque plus solution (Pharmacia) were seeded into plastic tissue culture dishes (Corning) to allow attachment of monocytes in PBMCs to the dishes. Then, the cells were cultured in the presence of 1000 IU/ml granulocyte macrophage colony-stimulating factor (R&D System) and 1000 IU/ml interleukin(IL)-4 (R&D System) for seven days. As medium, an AIM-V medium (Invitrogen) containing inactivated AB type serum (MP Biomedicals) was used (2% ABS/AIM-V medium). DCs induced to differentiate from monocytes by cytokines were pulsed with 20 micro-g/ml each of the synthesized peptides (37 degrees C., for three hours). Peptide pulsing was performed in the AIM-V medium. These peptide-pulsed DCs were inactivated by X-ray irradiation (20 Gy), and mixed in a 1:20 ratio with autologous CD8-positive T cells obtained by using the CD8 Positive Isolation Kit (Invitrogen) (1.5×10^4 DCs and 3×10^5 CD8-positive T cells), and cultured in a 48-well plate (Corning). The volume of 2% ABS/AIM-V medium was 0.5 ml per 1 well, and IL-7 (R&D System) was added (final concentration: 10 ng/ml) to the well. Two days after the start of culture, IL-2 (Novartis) was added thereto (final concentration: 20 IU/ml). On 7th day of culture and 14th day of culture, CD8-positive T cells were further stimulated with peptide-pulsed DCs. The DCs were prepared before use by the same method as above. On 21st day or thereafter (after the third DC stimulation), IFN-gamma production of CD8-positive T cells against peptide-pulsed C1R-A01 was confirmed by enzyme-linked immunospot (ELISPOT) assay (Tanaka H et al., Br J Cancer 2001, 84(1): 94-9; Umamo Y et al., Br J Cancer 2001, 84(8): 1052-7; Uchida N et al., Clin Cancer Res 2004, 10(24): 8577-86; Suda T et al., Cancer Sci 2006, 97(5): 411-9; Watanabe T et al., Cancer Sci 2005, 96(8): 498-506).

CTL Proliferation Procedure

[0487] CTLs were propagated using a method similar to that reported by Riddell et al. (Walter E A et al., N Engl J Med 1995, 333(16): 1038-44; Riddell SR et al., Nat Med 1996, 2(2): 216-23). In a flask for tissue culture (FALCON),

the CTLs were cultured in 5% ABS/AIM-V medium (culture solution volume: 25 ml/flask) with two types of human B lymphoblastoid cell lines (5×10^6 cells each) treated with mitomycin C and anti-CD3 antibody (BD biosciences, final concentration: 40 ng/ml). On the next day after the culturing was started, IL-2 was added to the culture (IL-2 final concentration: 120 IU/ml). On days 5, 8, and 11, the medium was changed to 5% ABS/AIM-V medium containing 60 IU/ml IL-2 (IL-2 final concentration: 30 IU/ml) (Tanaka H et al., Br J Cancer 2001, 84(1): 94-9; Umamo Y et al., Br J Cancer 2001, 84(8): 1052-7; Uchida N et al., Clin Cancer Res 2004, 10(24): 8577-86; Suda T et al., Cancer Sci 2006, 97(5): 411-9; Watanabe T et al., Cancer Sci 2005, 96(8): 498-506).

Establishment of CTL Clones (Limiting Dilution Method)

[0488] After CTLs were induced in vitro, the CTLs were seeded at 1 cell/well or 10 cells/well in a 96-well round-bottomed microplate (Corning). The CTLs were cultured in 5% ABS/AIM-V medium (culture solution volume: 150 μl /well) with two types of human B lymphoblastoid cell lines (1×10^4 cells each) treated with mitomycin C, anti-CD3 antibody (final concentration: 30 ng/ml), and IL-2 (final concentration: 125 Mimi). After 10 days, 50 μl of 5% ABS/AIM-V medium containing 500 IU/ml IL-2 was added to the culture. After 14th day, CTLs that showed peptide-specific IFN-gamma production in the ELISPOT assay were propagated by the method mentioned above (Uchida N et al., Clin Cancer Res 2004, 10(24): 8577-86; Suda T et al., Cancer Sci 2006, 97(5): 411-9; Watanabe T et al., Cancer Sci 2005, 96(8): 498-506).

Confirmation of IFN-Gamma Production

[0489] The IFN-gamma ELISPOT assay and IFN-gamma ELISA were performed to confirm peptide-specific IFN-gamma production of CTLs induced by a peptide. C1R-A01 pulsed with a peptide was prepared as target cells. The IFN-gamma ELISPOT assay and IFN-gamma ELISA were performed according to the procedures recommended by the assay kit manufacturer.

Establishment of Target Cells in which CDCA1 and HLA-A*01:01 were Forcibly Expressed

[0490] The cDNA encoding the CDCA1 or HLA-A*01:01 gene were amplified by PCR. Each PCR amplified product was incorporated into an expression vector. Using Lipofectamine 2000 (Invitrogen), either or both of the CDCA1 gene expression vector and the HLA-A*01:01 gene expression vector were introduced into COST cells, an HLA-negative cell line, according to the procedure recommended by the manufacturer. On the next day of the gene introduction, the COST cells were separated using Versene (Invitrogen) and used as target cells.

Results

[0491] Selection of HLA-A*01:01 binding peptides derived from CDCA1

[0492] Table 1a, Table 1 b, and Table 1c show 8 mer, 9 mer, and 10 mer peptides derived from CDCA1 that were predicted to bind to HLA-A*01:01 by “NetMHC 3.2” in descending order of binding affinity, respectively. Table 2a and Table 2b show 9 mer and 10 mer peptides derived from CDCA1 that were predicted to bind to HLA-A*01:01 by “BIMAS” in descending order of binding score. Table 3a

and Table 3b show 9 mer and 10 mer peptides derived from CDCA1 that were predicted to bind to HLA-A*01:01 by “SYFPEITHI” in descending order of binding score. The number of Start Position in each table indicates the position of the first amino acid of the peptide when counted from the N-terminus of the CDCA1 protein. Thirty-nine peptides in total potentially having HLA-A*01:01 binding ability became epitope peptide candidates.

TABLE 1a

CDCA1-derived 8mer peptide predicted to bind to HLA-A*01:01 by NetMHC3.2			
Start Position	Amino Acid Sequence	Affinity (nM)	SEQ ID NO
138	YMEFLWQY	50	1

TABLE 1b

CDCA1-derived 9mer peptides predicted to bind to HLA-A*01:01 by NetMHC3.2			
Start Position	Amino Acid Sequence	Affinity (nM)	SEQ ID NO
183	LSDGIQELQ	2486	2
49	YMRALQIVY	2570	3
325	ESDESELKK	3358	4
303	LSDNREKLA	6648	5
57	YGIRLEHFI	6700	6
380	VQEKRGAVY	8200	7

TABLE 1c

CDCA1-derived 10mer peptides predicted to bind to HLA-A*01:01 by NetMHC3.2			
Start Position	Amino Acid Sequence	Affinity (nM)	SEQ ID NO
136	ETymeFLWQY	92	8
66	MMPVNSEVMY	455	9
56	VYGIRLEHFI	1040	10
121	LSGIINHIHF	2410	11
287	PSCQLEVQLY	2630	12
48	IYMRALQIVY	2933	13
325	ESDESELKKL	4331	14
436	GIEKAEDSY	4636	15
379	KVQEKRGAVY	4867	16
129	HPREACRETY	7569	17
335	KTEENSPKRL	8751	18
424	LNLKTALEKY	9039	19

TABLE 1c-continued

CDCA1-derived 10mer peptides predicted to bind to HLA-A*01:01 by NetMHC3.2			
Start Position	Amino Acid Sequence	Affinity (nM)	SEQ ID NO
451	KTAELEKRMF	9261	20
146	KSSADKMQL	9961	21

TABLE 2a

CDCA1-derived 9mer peptides predicted to bind to HLA-A*01:01 by BIMAS			
Start Position	Amino Acid Sequence	Score	SEQ ID NO
335	KTEENSPKRL	112.5	22
41	KPEVLHMIY	56.25	23
25	GADGKNLTK	50	24
290	QLEVQLYQK	36	25
244	IVDSPEKLLK	10	26

TABLE 2b

CDCA1-derived 10mer peptides predicted to bind to HLA-A*01:01 by BIMAS			
Start Position	Amino Acid Sequence	Score	SEQ ID NO
452	TAELEKRMFK	90	27
91	HLDSFLPICR	25	28
323	QIESDESELK	18	29
290	QLEVQLYQKK	18	30
447	KIDEKTAELEK	10	31
106	TADILCPKAK	10	32

TABLE 3a

CDCA1-derived 9mer peptides predicted to bind to HLA-A*01:01 by SYFPEITHI			
Start Position	Amino Acid Sequence	Score	SEQ ID NO
130	FREACRETY	24	33
246	DSPEKLKNY	19	34
268	ARQEVVEKY	18	35
1	METLSFPRY	18	36
288	SCQLEVQLY	18	37

TABLE 3b

CDCA1-derived 10mer peptides predicted to bind to HLA-A*01:01 by SYFPEITHI			
Start Position	Amino Acid Sequence	Score	SEQ ID NO
303	LSDNREKLAS	18	38
245	VDSPEKLKNY	18	39

CTL Induction by HLA-A*01:01-Restricted CDCA1-Derived Peptides

[0493] CTLs specific to peptides derived from CDCA1 were induced according to the protocol described in “Materials and Methods.” Peptide-specific IFN-gamma production was measured by the IFN-gamma ELISPOT assay (FIG. 1). Peptide-specific IFN-gamma production relative to the control well was observed in:

- [0494]** Well #1 with CDCA1-A01-8-138 (SEQ ID NO: 1) (a),
- [0495]** Well #7 with CDCA1-A01-9-290 (SEQ ID NO: 25) (b),
- [0496]** Well #5 with CDCA1-A01-9-130 (SEQ ID NO: 33) (c),
- [0497]** Well #7 with CDCA1-A01-9-246 (SEQ ID NO: 34) (d),
- [0498]** Well #1 with CDCA1-A01-9-268 (SEQ ID NO: 35) (e),
- [0499]** Well #3 with CDCA1-A01-9-288 (SEQ ID NO: 37) (f),
- [0500]** Well #2 with CDCA1-A01-10-136 (SEQ ID NO: 8) (g),
- [0501]** Well #6 with CDCA1-A01-10-56 (SEQ ID NO: 10) (h), and
- [0502]** Well #6 with CDCA1-A01-10-48 (SEQ ID NO: 13) (i).

[0503] Meanwhile, no IFN-gamma production specific to other peptides shown in Table 1b, Table 1c, Table 2a, Table 2b, Table 3a, and Table 3b was observed. No IFN-gamma production specific to CDCA1-A01-10-66 (SEQ ID NO: 9), an example of typical negative data, was observed (j). Every peptide was likely to bind to HLA-A*01:01, but the nine peptides derived from CDCA1 were selected as peptides having strong CTL-inducing ability consequently.

Establishment of CTL Clones Specific to CDCA1-Derived HLA-A*01:01-Restricted Peptides

[0504] By limiting dilution method, CTL clones were established from cells in Well #2 which showed IFN-gamma production specific to CDCA1-A01-10-136 (SEQ ID NO: 8) (FIG. 1g), Well #6 which showed IFN-gamma production specific to CDCA1-A01-10-56 (SEQ ID NO: 10) (FIG. 1h), and Well #6 which showed IFN-gamma production specific to CDCA1-A01-10-48 (SEQ ID NO: 13) (FIG. 1i) in the IFN-gamma ELISPOT assay. As a result of IFN-gamma measurement by ELISA, CTL clones stimulated by CDCA1-A01-10-136 (SEQ ID NO: 8), CDCA1-A01-10-56 (SEQ ID NO: 10), or CDCA1-A01-10-48 (SEQ ID NO: 13) showed peptide-specific IFN-gamma production (FIG. 2).

IFN-Gamma Production of CTLs Against Target Cells Expressing CDCA1 and HLA-A*01:01

[0505] IFN-gamma production of CTL clones specific to CDCA1-A01-10-136 (SEQ ID NO: 8) against target cells expressing CDCA1 and HLA-A*01:01 was verified. COS7 cells expressing both CDCA1 and HLA-A*01:01 were prepared as target cells. COS7 cells expressing either CDCA1 or HLA-A*01:01 were prepared as negative control cells. CTL clones specific to CDCA1-A01-10-136 (SEQ ID NO: 8) showed IFN-gamma production against COS7 cells expressing both CDCA1 and HLA-A*01:01 (FIG. 3). By contrast, no significant IFN-gamma production was observed against negative control cells. From the above, it was clearly demonstrated that CDCA1-A01-10-136 (SEQ ID NO: 8) is a peptide produced by antigen processing and is presented on the cell surface together with the HLA-A01 molecule and recognized by CTLs. The result suggests that CDCA1-A01-10-136 (SEQ ID NO: 8) is useful as a cancer vaccine targeting cancer patients with elevated CDCA1 expression.

Homology Analysis of Antigen Peptides

[0506] It was confirmed that CDCA1-A01-8-138 (SEQ ID NO: 1), CDCA1-A01-9-290 (SEQ ID NO: 25), CDCA1-A01-9-130 (SEQ ID NO: 33), CDCA1-A01-9-246 (SEQ ID NO: 34), CDCA1-A01-9-268 (SEQ ID NO: 35), CDCA1-A01-9-288 (SEQ ID NO: 37), CDCA1-A01-10-136 (SEQ ID NO: 8), CDCA1-A01-10-56 (SEQ ID NO: 10), and CDCA1-A01-10-48 (SEQ ID NO: 13) were able to induce CTLs that showed peptide-specific IFN-gamma production. Thus, in order to confirm that the sequences of CDCA1-A01-8-138 (SEQ ID NO: 1), CDCA1-A01-9-290 (SEQ ID NO: 25), CDCA1-A01-9-130 (SEQ ID NO: 33), CDCA1-A01-9-246 (SEQ ID NO: 34), CDCA1-A01-9-268 (SEQ ID NO: 35), CDCA1-A01-9-288 (SEQ ID NO: 37), CDCA1-A01-10-136 (SEQ ID NO: 8), CDCA1-A01-10-56 (SEQ ID NO: 10), and CDCA1-A01-10-48 (SEQ ID NO: 13) are derived only from CDCA1, the homology analysis of the peptide sequences was performed using the BLAST algorithm (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The result shows that the sequences of CDCA1-A01-8-138 (SEQ ID NO: 1), CDCA1-A01-9-290 (SEQ ID NO: 25), CDCA1-A01-9-130 (SEQ ID NO: 33), CDCA1-A01-9-246 (SEQ ID NO: 34), CDCA1-A01-9-268 (SEQ ID NO: 35), CDCA1-A01-9-288 (SEQ ID NO: 37), CDCA1-A01-10-136 (SEQ ID NO: 8), CDCA1-A01-10-56 (SEQ ID NO: 10), and CDCA1-A01-10-48 (SEQ ID NO: 13) were observed only in CDCA1. Therefore, to the best of the present inventors' knowledge, these peptides are unique to CDCA1 and considered to have little possibility of eliciting an unintended immune response against molecules other than CDCA1 that are already known to sensitize the human immune system. In conclusion, novel HLA-A*01:01-restrictive epitope peptides derived from CDCA1 were identified and shown to be applicable to cancer immunotherapy.

INDUSTRIAL APPLICABILITY

[0507] The present invention provides CDCA 1-derived novel HLA-A01-restricted epitope peptides that induce a potent and specific anti-tumor immune response and thus have applicability for a wide range of cancer types. The peptides, compositions, APCs, and CTLs in the present invention can be used as a peptide vaccine for cancer

expressing CDCA1, for example, bladder cancer, breast cancer, cervical cancer, cholangiocellular cancer, chronic myeloid leukemia (CML), esophagus cancer, gastric cancer, non-small-cell lung cancer, lymphoma, osteosarcoma, prostate cancer, kidney cancer, small-cell lung cancer, head and neck cancer, soft tissue tumor, and colon cancer.

[0508] While the present invention is herein described in detail and with respect to specific embodiments thereof, it is

to be understood that the foregoing description is exemplary and explanatory in nature and is intended to illustrate the present invention and its preferred embodiments. Through routine experimentation, one skilled in the art will readily recognize that various changes and modifications can be made therein without departing from the spirit and scope of the present invention, the metes and bounds of which are defined by the appended claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 48

<210> SEQ ID NO 1

<211> LENGTH: 8

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 1

Tyr Met Glu Phe Leu Trp Gln Tyr
1 5

<210> SEQ ID NO 2

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 2

Leu Ser Asp Gly Ile Gln Glu Leu Gln
1 5

<210> SEQ ID NO 3

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 3

Tyr Met Arg Ala Leu Gln Ile Val Tyr
1 5

<210> SEQ ID NO 4

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 4

Glu Ser Asp Glu Ser Glu Leu Lys Lys
1 5

<210> SEQ ID NO 5

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 5

Leu Ser Asp Asn Arg Glu Lys Leu Ala

-continued

1 5

<210> SEQ ID NO 6
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 6

Tyr Gly Ile Arg Leu Glu His Phe Tyr
1 5

<210> SEQ ID NO 7
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 7

Val Gln Glu Lys Arg Gly Ala Val Tyr
1 5

<210> SEQ ID NO 8
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 8

Glu Thr Tyr Met Glu Phe Leu Trp Gln Tyr
1 5 10

<210> SEQ ID NO 9
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 9

Met Met Pro Val Asn Ser Glu Val Met Tyr
1 5 10

<210> SEQ ID NO 10
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 10

Val Tyr Gly Ile Arg Leu Glu His Phe Tyr
1 5 10

<210> SEQ ID NO 11
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 11

-continued

Leu Ser Gly Ile Ile Asn Phe Ile His Phe
1 5 10

<210> SEQ ID NO 12
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 12

Pro Ser Cys Gln Leu Glu Val Gln Leu Tyr
1 5 10

<210> SEQ ID NO 13
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 13

Ile Tyr Met Arg Ala Leu Gln Ile Val Tyr
1 5 10

<210> SEQ ID NO 14
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 14

Glu Ser Asp Glu Ser Glu Leu Lys Lys Leu
1 5 10

<210> SEQ ID NO 15
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 15

Gly Ile Glu Lys Ala Ala Glu Asp Ser Tyr
1 5 10

<210> SEQ ID NO 16
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 16

Lys Val Gln Glu Lys Arg Gly Ala Val Tyr
1 5 10

<210> SEQ ID NO 17
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

-continued

<400> SEQUENCE: 17

His	Phe	Arg	Glu	Ala	Cys	Arg	Glu	Thr	Tyr
1				5					10

<210> SEQ ID NO 18

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 18

Lys	Thr	Glu	Glu	Asn	Ser	Phe	Lys	Arg	Leu
1				5					10

<210> SEQ ID NO 19

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 19

Leu	Asn	Leu	Lys	Thr	Ala	Leu	Glu	Lys	Tyr
1				5					10

<210> SEQ ID NO 20

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 20

Lys	Thr	Ala	Glu	Leu	Lys	Arg	Lys	Met	Phe
1				5					10

<210> SEQ ID NO 21

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 21

Lys	Ser	Ser	Ala	Asp	Lys	Met	Gln	Gln	Leu
1				5					10

<210> SEQ ID NO 22

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 22

Lys	Thr	Glu	Glu	Asn	Ser	Phe	Lys	Arg
1				5				

<210> SEQ ID NO 23

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 23

Lys Pro Glu Val Leu His Met Ile Tyr
1 5

<210> SEQ ID NO 24

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 24

Gly Ala Asp Gly Lys Asn Leu Thr Lys
1 5

<210> SEQ ID NO 25

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 25

Gln Leu Glu Val Gln Leu Tyr Gln Lys
1 5

<210> SEQ ID NO 26

<211> LENGTH: 9

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 26

Ile Val Asp Ser Pro Glu Lys Leu Lys
1 5

<210> SEQ ID NO 27

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 27

Thr Ala Glu Leu Lys Arg Lys Met Phe Lys
1 5 10

<210> SEQ ID NO 28

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 28

His Leu Asp Ser Phe Leu Pro Ile Cys Arg
1 5 10

<210> SEQ ID NO 29

<211> LENGTH: 10

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 29

Gln Ile Glu Ser Asp Glu Ser Glu Leu Lys
1 5 10

<210> SEQ ID NO 30
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 30

Gln Leu Glu Val Gln Leu Tyr Gln Lys Lys
1 5 10

<210> SEQ ID NO 31
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 31

Lys Ile Asp Glu Lys Thr Ala Glu Leu Lys
1 5 10

<210> SEQ ID NO 32
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 32

Thr Ala Asp Ile Leu Cys Pro Lys Ala Lys
1 5 10

<210> SEQ ID NO 33
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 33

Phe Arg Glu Ala Cys Arg Glu Thr Tyr
1 5

<210> SEQ ID NO 34
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 34

Asp Ser Pro Glu Lys Leu Lys Asn Tyr
1 5

-continued

<210> SEQ ID NO 35
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 35

Ala Arg Gln Glu Val Val Glu Lys Tyr
1 5

<210> SEQ ID NO 36
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 36

Met Glu Thr Leu Ser Phe Pro Arg Tyr
1 5

<210> SEQ ID NO 37
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 37

Ser Cys Gln Leu Glu Val Gln Leu Tyr
1 5

<210> SEQ ID NO 38
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 38

Leu Ser Asp Asn Arg Glu Lys Leu Ala Ser
1 5 10

<210> SEQ ID NO 39
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a peptide derived from CDCA1

<400> SEQUENCE: 39

Val Asp Ser Pro Glu Lys Leu Lys Asn Tyr
1 5 10

<210> SEQ ID NO 40
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a PCR primer for the TCR analysis

<400> SEQUENCE: 40

gtctaccagg cattcgcttc at

-continued

```

<210> SEQ ID NO 41
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a PCR primer for the TCR analysis

<400> SEQUENCE: 41

tcagctggac cacagccgca gcgt                                24

<210> SEQ ID NO 42
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a PCR primer for the TCR analysis

<400> SEQUENCE: 42

tcagaaatcc tttctcttga c                                    21

<210> SEQ ID NO 43
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a PCR primer for the TCR analysis

<400> SEQUENCE: 43

ctagcctctg gaatcctttc tctt                                24

<210> SEQ ID NO 44
<211> LENGTH: 1989
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (301)..(1695)

<400> SEQUENCE: 44

gcggaatggg gcgggacttc cagtaggagg cggaagttt gaaaagtgat gacgggtgac      60
gtttgctgat ttttgacttt gctttagct gctccccgaa ctgcgcgtct tcctgtcggc      120
ggccggcact gtaggtagc gcgagaggac ggaggaagga agcctgcaga cagacgcctt      180
ctccatcca aggcgcgggc aggtgccggg acgctgggcc tggcgggtgt ttcgtcgtgc      240
tcagcgggtgg gaggaggcgg aagaaaccag agcctgggag attaacagga aacttccaag      300
atg gaa act ttg tct ttc ccc aga tat aat gta gct gag att gtg att      348
Met Glu Thr Leu Ser Phe Pro Arg Tyr Asn Val Ala Glu Ile Val Ile
1          5          10         15

cat att cgc aat aag atc tta aca gga gct gat ggt aaa aac ctg acc      396
His Ile Arg Asn Lys Ile Leu Thr Gly Ala Asp Gly Lys Asn Leu Thr
          20         25         30

aag aat gat ctt tat cca aat cca aag cct gaa gtc ttg cac atg atc      444
Lys Asn Asp Leu Tyr Pro Asn Pro Lys Pro Glu Val Leu His Met Ile
          35         40         45

tac atg aga gcc tta caa ata gta tat gga att cga ctg gaa cat ttt      492
Tyr Met Arg Ala Leu Gln Ile Val Tyr Gly Ile Arg Leu Glu His Phe
          50         55         60

tac atg atg cca gtg aac tct gaa gtc atg tat cca cat tta atg gaa      540
Tyr Met Met Pro Val Asn Ser Glu Val Met Tyr Pro His Leu Met Glu
          65         70         75         80

ggc ttc tta cca ttc agc aat tta gtt act cat ctg gac tca ttt ttg      588
Gly Phe Leu Pro Phe Ser Asn Leu Val Thr His Leu Asp Ser Phe Leu
          85         90         95

```

-continued

cct atc tgc cgg gtg aat gac ttt gag act gct gat att cta tgt cca	636
Pro Ile Cys Arg Val Asn Asp Phe Glu Thr Ala Asp Ile Leu Cys Pro	
100 105 110	
aaa gca aaa cgg aca agt cgg ttt tta agt ggc att atc aac ttt att	684
Lys Ala Lys Arg Thr Ser Arg Phe Leu Ser Gly Ile Ile Asn Phe Ile	
115 120 125	
cac ttc aga gaa gca tgc cgt gaa acg tat atg gaa ttt ctt tgg caa	732
His Phe Arg Glu Ala Cys Arg Glu Thr Tyr Met Glu Phe Leu Trp Gln	
130 135 140	
tat aaa tcc tct gcg gac aaa atg caa cag tta aac gcc gca cac cag	780
Tyr Lys Ser Ser Ala Asp Lys Met Gln Gln Leu Asn Ala Ala His Gln	
145 150 155 160	
gag gca tta atg aaa ctg gag aga ctt gat tct gtt cca gtt gaa gag	828
Glu Ala Leu Met Lys Leu Glu Arg Leu Asp Ser Val Pro Val Glu Glu	
165 170 175	
caa gaa gag ttc aag cag ctt tca gat gga att cag gag cta caa caa	876
Gln Glu Glu Phe Lys Gln Leu Ser Asp Gly Ile Gln Glu Leu Gln Gln	
180 185 190	
tca cta aat cag gat ttt cat caa aaa acg ata gtg ctg caa gag gga	924
Ser Leu Asn Gln Asp Phe His Gln Lys Thr Ile Val Leu Gln Glu Gly	
195 200 205	
aat tcc caa aag aag tca aat att tca gag aaa acc aag cgt ttg aat	972
Asn Ser Gln Lys Lys Ser Asn Ile Ser Glu Lys Thr Lys Arg Leu Asn	
210 215 220	
gaa cta aaa ttg tcg gtg gtt tct ttg aaa gaa ata caa gag agt ttg	1020
Glu Leu Lys Leu Ser Val Val Ser Leu Lys Glu Ile Gln Glu Ser Leu	
225 230 235 240	
aaa aca aaa att gtg gat tct cca gag aag tta aag aat tat aaa gaa	1068
Lys Thr Lys Ile Val Asp Ser Pro Glu Lys Leu Lys Asn Tyr Lys Glu	
245 250 255	
aaa atg aaa gat acg gtc cag aag ctt aaa aat gcc aga caa gaa gtg	1116
Lys Met Lys Asp Thr Val Gln Lys Leu Lys Asn Ala Arg Gln Glu Val	
260 265 270	
gtg gag aaa tat gaa atc tat gga gac tca gtt gac tgc ctg cct tca	1164
Val Glu Lys Tyr Glu Ile Tyr Gly Asp Ser Val Asp Cys Leu Pro Ser	
275 280 285	
tgt cag ttg gaa gtg cag tta tat caa aag aaa ata cag gac ctt tca	1212
Cys Gln Leu Glu Val Gln Leu Tyr Gln Lys Lys Ile Gln Asp Leu Ser	
290 295 300	
gat aat agg gaa aaa tta gcc agt atc tta aag gag agc ctg aac ttg	1260
Asp Asn Arg Glu Lys Leu Ala Ser Ile Leu Lys Glu Ser Leu Asn Leu	
305 310 315 320	
gag gac caa att gag agt gat gag tca gaa ctg aag aaa ttg aag act	1308
Glu Asp Gln Ile Glu Ser Asp Glu Ser Glu Leu Lys Lys Leu Lys Thr	
325 330 335	
gaa gaa aat tcg ttc aaa aga ctg atg att gtg aag aag gaa aaa ctt	1356
Glu Glu Asn Ser Phe Lys Arg Leu Met Ile Val Lys Lys Glu Lys Leu	
340 345 350	
gcc aca gca caa ttc aaa ata aat aag aag cat gaa gat gtt aag caa	1404
Ala Thr Ala Gln Phe Lys Ile Asn Lys Lys His Glu Asp Val Lys Gln	
355 360 365	
tac aaa cgc aca gta att gag gat tgc aat aaa gtt caa gaa aaa aga	1452
Tyr Lys Arg Thr Val Ile Glu Asp Cys Asn Lys Val Gln Glu Lys Arg	
370 375 380	
ggt gct gtc tat gaa cga gta acc aca att aat caa gaa atc caa aaa	1500
Gly Ala Val Tyr Glu Arg Val Thr Thr Ile Asn Gln Glu Ile Gln Lys	
385 390 395 400	

-continued

att aaa ctt gga att caa caa cta aaa gat gct gct gaa agg gag aaa	1548
Ile Lys Leu Gly Ile Gln Gln Leu Lys Asp Ala Ala Glu Arg Glu Lys	
405 410 415	
ctg aag tcc cag gaa ata ttt cta aac ttg aaa act gct ttg gag aaa	1596
Leu Lys Ser Gln Glu Ile Phe Leu Asn Leu Lys Thr Ala Leu Glu Lys	
420 425 430	
tac cac gac ggt att gaa aag gca gca gag gac tcc tat gct aag ata	1644
Tyr His Asp Gly Ile Glu Lys Ala Ala Glu Asp Ser Tyr Ala Lys Ile	
435 440 445	
gat gag aag aca gct gaa ctg aag agg aag atg ttc aaa atg tca acc	1692
Asp Glu Lys Thr Ala Glu Leu Lys Arg Lys Met Phe Lys Met Ser Thr	
450 455 460	
tga ttaacaaaaat tacatgtctt ttgttaaag gcttgccatc ttttaatttt	1745
ctatttagaa agaaaagttg aagcgaatgg aagtatcaga agtaccaaat aatggtggct	1805
tcatacagttt ttatacactc tcataagtag ttaataagat gaatttaagtaggctttta	1865
ttatatttata attaaaaataa cttgtgcagc tattcatgtc tctactctgc ccttgtttgt	1925
aaatagtttg agtaaaacaa aactagttac ctttgaaata tataatatttt tttctgttac	1985
tatc	1989

<210> SEQ ID NO 45

<211> LENGTH: 464

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 45

Met Glu Thr Leu Ser Phe Pro Arg Tyr Asn Val Ala Glu Ile Val Ile
1 5 10 15
His Ile Arg Asn Lys Ile Leu Thr Gly Ala Asp Gly Lys Asn Leu Thr
20 25 30
Lys Asn Asp Leu Tyr Pro Asn Pro Lys Pro Glu Val Leu His Met Ile
35 40 45
Tyr Met Arg Ala Leu Gln Ile Val Tyr Gly Ile Arg Leu Glu His Phe
50 55 60
Tyr Met Met Pro Val Asn Ser Glu Val Met Tyr Pro His Leu Met Glu
65 70 75 80
Gly Phe Leu Pro Phe Ser Asn Leu Val Thr His Leu Asp Ser Phe Leu
85 90 95
Pro Ile Cys Arg Val Asn Asp Phe Glu Thr Ala Asp Ile Leu Cys Pro
100 105 110
Lys Ala Lys Arg Thr Ser Arg Phe Leu Ser Gly Ile Ile Asn Phe Ile
115 120 125
His Phe Arg Glu Ala Cys Arg Glu Thr Tyr Met Glu Phe Leu Trp Gln
130 135 140
Tyr Lys Ser Ser Ala Asp Lys Met Gln Gln Leu Asn Ala Ala His Gln
145 150 155 160
Glu Ala Leu Met Lys Leu Glu Arg Leu Asp Ser Val Pro Val Glu Glu
165 170 175
Gln Glu Glu Phe Lys Gln Leu Ser Asp Gly Ile Gln Glu Leu Gln Gln
180 185 190
Ser Leu Asn Gln Asp Phe His Gln Lys Thr Ile Val Leu Gln Glu Gly
195 200 205
Asn Ser Gln Lys Lys Ser Asn Ile Ser Glu Lys Thr Lys Arg Leu Asn
210 215 220
Glu Leu Lys Leu Ser Val Val Ser Leu Lys Glu Ile Gln Glu Ser Leu
225 230 235 240
Lys Thr Lys Ile Val Asp Ser Pro Glu Lys Leu Lys Asn Tyr Lys Glu

-continued

245										250										255									
Lys	Met	Lys	Asp	Thr	Val	Gln	Lys	Leu	Lys	Asn	Ala	Arg	Gln	Glu	Val														
			260					265					270																
Val	Glu	Lys	Tyr	Glu	Ile	Tyr	Gly	Asp	Ser	Val	Asp	Cys	Leu	Pro	Ser														
			275				280					285																	
Cys	Gln	Leu	Glu	Val	Gln	Leu	Tyr	Gln	Lys	Lys	Ile	Gln	Asp	Leu	Ser														
			290			295					300																		
Asp	Asn	Arg	Glu	Lys	Leu	Ala	Ser	Ile	Leu	Lys	Glu	Ser	Leu	Asn	Leu														
					310					315				320															
Glu	Asp	Gln	Ile	Glu	Ser	Asp	Glu	Ser	Glu	Leu	Lys	Lys	Leu	Lys	Thr														
				325					330					335															
Glu	Glu	Asn	Ser	Phe	Lys	Arg	Leu	Met	Ile	Val	Lys	Lys	Glu	Lys	Leu														
				340				345					350																
Ala	Thr	Ala	Gln	Phe	Lys	Ile	Asn	Lys	Lys	His	Glu	Asp	Val	Lys	Gln														
			355				360				365																		
Tyr	Lys	Arg	Thr	Val	Ile	Glu	Asp	Cys	Asn	Lys	Val	Gln	Glu	Lys	Arg														
			370			375					380																		
Gly	Ala	Val	Tyr	Glu	Arg	Val	Thr	Thr	Ile	Asn	Gln	Glu	Ile	Gln	Lys														
					390					395				400															
Ile	Lys	Leu	Gly	Ile	Gln	Gln	Leu	Lys	Asp	Ala	Ala	Glu	Arg	Glu	Lys														
				405					410					415															
Leu	Lys	Ser	Gln	Glu	Ile	Phe	Leu	Asn	Leu	Lys	Thr	Ala	Leu	Glu	Lys														
				420				425					430																
Tyr	His	Asp	Gly	Ile	Glu	Lys	Ala	Ala	Glu	Asp	Ser	Tyr	Ala	Lys	Ile														
			435				440				445																		
Asp	Glu	Lys	Thr	Ala	Glu	Leu	Lys	Arg	Lys	Met	Phe	Lys	Met	Ser	Thr														
			450			455					460																		
<210> SEQ ID NO 46																													
<211> LENGTH: 1843																													
<212> TYPE: DNA																													
<213> ORGANISM: Homo sapiens																													
<220> FEATURE:																													
<221> NAME/KEY: CDS																													
<222> LOCATION: (155)..(1549)																													
<400> SEQUENCE: 46																													
g																													
c																													
g																													
g																													
a																													
a																													
t																													
t																													
g																													
c																													
a																													
g																													
g																													
c																													
t																													
g																													
a																													
c																													
t																													
t																													
c																													
c																													
c																													
t																													
t																													
c																													
c																													
t																													
t																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
t																													
t																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													
t																													
g																													
a																													
a																													
g																													
a																													
t																													
t																													
g																													
c																													
c																													
t																													

-continued

75	80	85	
tta gtt act cat ctg gac	tca ttt ttg cct atc	tgc cgg gtg aat gac	463
Leu Val Thr His Leu Asp	Ser Phe Leu Pro Ile	Cys Arg Val Asn Asp	
90	95	100	
ttt gag act gct gat att	cta tgt cca aaa gca	aaa cgg aca agt cgg	511
Phe Glu Thr Ala Asp Ile	Leu Cys Pro Lys Ala	Lys Arg Thr Ser Arg	
105	110	115	
ttt tta agt ggc att atc	aac ttt att cac ttc	aga gaa gca tgc cgt	559
Phe Leu Ser Gly Ile Ile	Asn Phe Ile His Phe	Arg Glu Ala Cys Arg	
120	125	130	135
gaa acg tat atg gaa ttt	ctt tgg caa tat aaa	tcc tct gcg gac aaa	607
Glu Thr Tyr Met Phe Leu	Trp Gln Tyr Lys Ser	Ser Ala Asp Lys	
140	145	150	
atg caa cag tta aac gcc	gca cac cag gag gca	tta atg aaa ctg gag	655
Met Gln Gln Leu Asn Ala	Ala His Gln Glu Ala	Leu Met Lys Leu Glu	
155	160	165	
aga ctt gat tct gtt cca	gtt gaa gag caa gaa	gag ttc aag cag ctt	703
Arg Leu Asp Ser Val Pro	Val Glu Glu Gln Glu	Glu Phe Lys Gln Leu	
170	175	180	
tca gat gga att cag gag	cta caa caa tca cta	aat cag gat ttt cat	751
Ser Asp Gly Ile Gln Glu	Leu Gln Gln Ser Leu	Asn Gln Asp Phe His	
185	190	195	
caa aaa acg ata gtg ctg	caa gag gga aat tcc	caa aag aag tca aat	799
Gln Lys Thr Ile Val Leu	Gln Glu Gly Asn Ser	Gln Lys Lys Ser Asn	
200	205	210	215
att tca gag aaa acc aag	cgt ttg aat gaa cta	aaa ttg tcg gtg gtt	847
Ile Ser Glu Lys Thr Lys	Arg Leu Asn Glu Leu	Lys Leu Ser Val Val	
220	225	230	
tct ttg aaa gaa ata caa	gag agt ttg aaa aca	aaa att gtg gat tct	895
Ser Leu Lys Glu Ile Gln	Glu Ser Leu Lys Thr	Lys Ile Val Asp Ser	
235	240	245	
cca gag aag tta aag aat	tat aaa gaa aaa atg	aaa gat acg gtc cag	943
Pro Glu Lys Leu Lys Asn	Tyr Lys Glu Lys Met	Lys Asp Thr Val Gln	
250	255	260	
aag ctt aaa aat gcc aga	caa gaa gtg gtg gag	aaa tat gaa atc tat	991
Lys Leu Lys Asn Ala Arg	Gln Glu Val Val Glu	Lys Tyr Glu Ile Tyr	
265	270	275	
gga gac tca gtt gac tgc	ctg cct tca tgt cag	ttg gaa gtg cag tta	1039
Gly Asp Ser Val Asp Cys	Leu Pro Ser Cys Gln	Leu Glu Val Gln Leu	
280	285	290	295
tat caa aag aaa ata cag	gac ctt tca gat aat	agg gaa aaa tta gcc	1087
Tyr Gln Lys Lys Ile Gln	Asp Leu Ser Asp Asn	Arg Glu Lys Leu Ala	
300	305	310	
agt atc tta aag gag agc	ctg aac ttg gag gac	caa att gag agt gat	1135
Ser Ile Leu Lys Glu Ser	Leu Asn Leu Glu Asp	Gln Ile Glu Ser Asp	
315	320	325	
gag tca gaa ctg aag aaa	ttg aag act gaa gaa	aat tcg ttc aaa aga	1183
Glu Ser Glu Leu Lys Lys	Leu Lys Thr Glu Glu	Asn Ser Phe Lys Arg	
330	335	340	
ctg atg att gtg aag aag	gaa aaa ctt gcc aca	gca caa ttc aaa ata	1231
Leu Met Ile Val Lys Lys	Glu Lys Leu Ala Thr	Ala Gln Phe Lys Ile	
345	350	355	
aat aag aag cat gaa gat	gtt aag caa tac aaa	cgc aca gta att gag	1279
Asn Lys Lys His Glu Asp	Val Lys Gln Tyr Lys	Arg Thr Val Ile Glu	
360	365	370	375
gat tgc aat aaa gtt caa	gaa aaa aga ggt gct	gtc tat gaa cga gta	1327
Asp Cys Asn Lys Val Gln	Glu Lys Arg Gly Ala	Val Tyr Glu Arg Val	

-continued

380	385	390	
acc aca att aat caa gaa atc caa aaa att aaa ctt gga att caa caa			1375
Thr Thr Ile Asn Gln Glu Ile Gln Lys Ile Lys Leu Gly Ile Gln Gln			
395	400	405	
cta aaa gat gct gct gaa agg gag aaa ctg aag tcc cag gaa ata ttt			1423
Leu Lys Asp Ala Ala Glu Arg Glu Lys Leu Lys Ser Gln Glu Ile Phe			
410	415	420	
cta aac ttg aaa act gct ttg gag aaa tac cac gac ggt att gaa aag			1471
Leu Asn Leu Lys Thr Ala Leu Glu Lys Tyr His Asp Gly Ile Glu Lys			
425	430	435	
gca gca gag gac tcc tat gct aag ata gat gag aag aca gct gaa ctg			1519
Ala Ala Glu Asp Ser Tyr Ala Lys Ile Asp Glu Lys Thr Ala Glu Leu			
440	445	450	455
aag agg aag atg ttc aaa atg tca acc tga ttaacaaaat tacatgtctt			1569
Lys Arg Lys Met Phe Lys Met Ser Thr			
460			
tttgtaaatg gcttgccatc ttttaatttt ctatttagaa agaaaagtgg aagcgaatgg			1629
aagtatcaga agtaccaaat aatgttggtt tcatcagttt ttatacactc tcataagtag			1689
ttataaagat gaatttaatg taggctttta ttaatttata attaaaaataa cttgtgcagc			1749
tattcatgtc tctactctgc cccttggtgt aaatagtttg agtaaaacaa aactagttac			1809
ctttgaaata tataatatttt tttctgttac tate			1843
<210> SEQ ID NO 47			
<211> LENGTH: 464			
<212> TYPE: PRT			
<213> ORGANISM: Homo sapiens			
<400> SEQUENCE: 47			
Met Glu Thr Leu Ser Phe Pro Arg Tyr Asn Val Ala Glu Ile Val Ile			
1	5	10	15
His Ile Arg Asn Lys Ile Leu Thr Gly Ala Asp Gly Lys Asn Leu Thr			
20	25	30	
Lys Asn Asp Leu Tyr Pro Asn Pro Lys Pro Glu Val Leu His Met Ile			
35	40	45	
Tyr Met Arg Ala Leu Gln Ile Val Tyr Gly Ile Arg Leu Glu His Phe			
50	55	60	
Tyr Met Met Pro Val Asn Ser Glu Val Met Tyr Pro His Leu Met Glu			
65	70	75	80
Gly Phe Leu Pro Phe Ser Asn Leu Val Thr His Leu Asp Ser Phe Leu			
85	90	95	
Pro Ile Cys Arg Val Asn Asp Phe Glu Thr Ala Asp Ile Leu Cys Pro			
100	105	110	
Lys Ala Lys Arg Thr Ser Arg Phe Leu Ser Gly Ile Ile Asn Phe Ile			
115	120	125	
His Phe Arg Glu Ala Cys Arg Glu Thr Tyr Met Glu Phe Leu Trp Gln			
130	135	140	
Tyr Lys Ser Ser Ala Asp Lys Met Gln Gln Leu Asn Ala Ala His Gln			
145	150	155	160
Glu Ala Leu Met Lys Leu Glu Arg Leu Asp Ser Val Pro Val Glu Glu			
165	170	175	
Gln Glu Glu Phe Lys Gln Leu Ser Asp Gly Ile Gln Glu Leu Gln Gln			
180	185	190	
Ser Leu Asn Gln Asp Phe His Gln Lys Thr Ile Val Leu Gln Glu Gly			
195	200	205	
Asn Ser Gln Lys Lys Ser Asn Ile Ser Glu Lys Thr Lys Arg Leu Asn			
210	215	220	

-continued

```

Glu Leu Lys Leu Ser Val Val Ser Leu Lys Glu Ile Gln Glu Ser Leu
225                230                235                240

Lys Thr Lys Ile Val Asp Ser Pro Glu Lys Leu Lys Asn Tyr Lys Glu
                245                250                255

Lys Met Lys Asp Thr Val Gln Lys Leu Lys Asn Ala Arg Gln Glu Val
                260                265                270

Val Glu Lys Tyr Glu Ile Tyr Gly Asp Ser Val Asp Cys Leu Pro Ser
                275                280                285

Cys Gln Leu Glu Val Gln Leu Tyr Gln Lys Lys Ile Gln Asp Leu Ser
                290                295                300

Asp Asn Arg Glu Lys Leu Ala Ser Ile Leu Lys Glu Ser Leu Asn Leu
305                310                315                320

Glu Asp Gln Ile Glu Ser Asp Glu Ser Glu Leu Lys Lys Leu Lys Thr
                325                330                335

Glu Glu Asn Ser Phe Lys Arg Leu Met Ile Val Lys Lys Glu Lys Leu
                340                345                350

Ala Thr Ala Gln Phe Lys Ile Asn Lys Lys His Glu Asp Val Lys Gln
                355                360                365

Tyr Lys Arg Thr Val Ile Glu Asp Cys Asn Lys Val Gln Glu Lys Arg
370                375                380

Gly Ala Val Tyr Glu Arg Val Thr Thr Ile Asn Gln Glu Ile Gln Lys
385                390                395                400

Ile Lys Leu Gly Ile Gln Gln Leu Lys Asp Ala Ala Glu Arg Glu Lys
                405                410                415

Leu Lys Ser Gln Glu Ile Phe Leu Asn Leu Lys Thr Ala Leu Glu Lys
                420                425                430

Tyr His Asp Gly Ile Glu Lys Ala Ala Glu Asp Ser Tyr Ala Lys Ile
                435                440                445

Asp Glu Lys Thr Ala Glu Leu Lys Arg Lys Met Phe Lys Met Ser Thr
450                455                460

```

```

<210> SEQ ID NO 48
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: a linker peptide

```

```

<400> SEQUENCE: 48

```

```

Asn Lys Arg Lys
1

```

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

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

2. The peptide of claim 1, comprising the amino acid sequence in which one or more substitution(s) selected from

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

- (a) the second amino acid from the N terminus is substituted with an amino acid selected from the group consisting of threonine and serine;
- (b) the third amino acid from the N terminus is substituted with an amino acid selected from the group consisting of aspartic acid and glutamic acid; and
- (c) the C-terminal amino acid is substituted with tyrosine.

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

4. An isolated polynucleotide, which encodes the peptide of claim 1.

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

- (a) one or more types of peptides of claim 1;
- (b) one or more types of polynucleotides encoding the peptide(s) of claim 1 in an expressible form;
- (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of claim 1 and an HLA antigen;
- (d) an exosome that presents on its cell surface a complex of the peptide of claim 1 and an HLA antigen; and
- (e) a CTL that targets the peptide of claim 1.

6. A composition for inducing a CTL(s), comprising a pharmaceutically acceptable carrier and at least one ingredient selected from the group consisting of (a) to (d) below:

- (a) one or more types of peptides of claim 1;
- (b) one or more types of polynucleotides encoding the peptide(s) of claim 1 in an expressible form;
- (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of claim 1 and an HLA antigen; and
- (d) an exosome that presents on its cell surface a complex of the peptide of claim 1 and an HLA antigen.

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

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

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

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

11. The composition of claim 5, which is formulated for administration to a subject positive for HLA-A01.

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

- (a) contacting an APC(s) with the peptide of claim 1 in vitro, ex vivo or in vivo; and
- (b) introducing a polynucleotide encoding the peptide of claim 1 into an APC(s).

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

- (a) co-culturing a CD8-positive T cell(s) with an APC(s) that presents on its surface a complex of an HLA antigen and the peptide of claim 1;
- (b) co-culturing a CD8-positive T cell(s) with an exosome (s) that presents on its surface a complex of an HLA antigen and the peptide of claim 1; and
- (c) introducing into a CD8-positive T cell(s) a polynucleotide encoding each subunit of a T cell receptor (TCR)

capable of binding to the peptide of claim 1 presented by an HLA antigen on a cell surface.

14. An isolated APC that presents on its surface a complex of an HLA antigen and the peptide of claim 1.

15. An isolated APC induced by the method of claim 12.

16. An isolated CTL that targets the peptide of claim 1.

17. An isolated CTL induced by the method of claim 13.

18. A method of inducing an immune response against cancer, which comprises administering to a subject at least one ingredient selected from the group consisting of (a) to (e) below:

- (a) one or more types of peptides of claim 1;
- (b) one or more types of polynucleotides encoding the peptide(s) of claim 1 in an expressible form;
- (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of claim 1 and an HLA antigen;
- (d) an exosome that presents on its cell surface a complex of the peptide of claim 1 and an HLA antigen; and
- (e) a CTL that targets the peptide of claim 1.

19. A method of treating and/or preventing cancer, and/or preventing postoperative recurrence thereof, which comprises administering to a subject at least one ingredient selected from the group consisting of (a) to (e) below:

- (a) one or more types of peptides of claim 1;
- (b) one or more types of polynucleotides encoding the peptide(s) of claim 1 in an expressible form;
- (c) an antigen-presenting cell (APC) that presents on its cell surface a complex of the peptide of claim 1 and an HLA antigen;
- (d) an exosome that presents on its cell surface a complex of the peptide of claim 1 and an HLA antigen; and
- (e) a CTL that targets the peptide of claim 1.

20. An antibody that binds to the peptide of claim 1.

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

- (a) generating candidate sequences consisting of an amino acid sequence in which one, two or several amino acid residues are substituted, deleted, inserted and/or added to an original amino acid sequence consisting of the amino acid sequence selected from among SEQ ID NOs: 1, 8, 10, 13, 25, 33 to 35 and 37;
- (b) selecting from among the candidate sequences generated in (a), a candidate sequence that does not have significant homology (sequence identity) with any known human gene product other than CDCA1;
- (c) contacting an APC with a peptide consisting of the candidate sequence selected in (b);
- (d) contacting the APC of (c) with a CD8-positive T cell; and
- (e) selecting a peptide having an equal to or higher CTL-inducing ability than that of a peptide consisting of the original amino acid sequence.

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

23. A kit comprising a container that houses the composition of claim 5 and a container that houses an adjuvant.

* * * * *