Description

Title of Invention: INHBB EPITOPE PEPTIDES AND VACCINES CONTAINING THE SAME

Technical Field

[0001] Priority

The present application claims the benefit of U.S. Provisional Application No. 61/089,973, filed on August 19, 2008, the entire content of which is incorporated by reference herein.

[0002] Technical Field

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 extremely effective as cancer vaccines, and drugs for treating and preventing tumors.

Background Art

[0003] It has been demonstrated that CD8 positive CTLs recognize epitope peptides derived from the tumor-associated antigens (TAAs) found on major histocompatibility complex (MHC) class I molecule, and then kill the tumor cells. Since the discovery of the melanoma antigen (MAGE) family as the first example of TAAs, many other TAAs have been discovered, primarily through immunological approaches (NPL 1: Boon T, Int J Cancer 1993 May 8, 54(2): 177-80; NPL 2: Boon T & van der Bruggen P, J Exp Med 1996 Mar 1, 183(3): 725-9). Some of the TAAs are now currently undergoing clinical development as immunotherapeutic targets.

[0004] Identification of new TAAs capable of inducing potent and specific anti-tumor immune responses, warrants further development and clinical application of peptide vaccination strategies for various types of cancer (NPL 3: Harris CC, J Natl Cancer Inst 1996 Oct 16, 88(20): 1442-55; NPL 4 : Butterfield LH et al., Cancer Res 1999 Jul 1, 59(13): 3134-42; NPL 5: Vissers JL et al., Cancer Res 1999 Nov 1, 59(21): 5554-9; NPL 6: van der Burg SH et al., J Immunol 1996 May 1, 156(9): 3308-14; NPL 7: Tanaka F et al., Cancer Res 1997 Oct 15, 57(20): 4465-8; NPL 8: Fujie T et al., Int J Cancer 1999 Jan 18, 80(2): 169-72; NPL 9: Kikuchi M et al., Int J Cancer 1999 May 5, 81(3): 459-66; NPL 10: Oiso M et al., Int J Cancer 1999 May 5, 81(3): 387-94). To date, there have been several reports of clinical trials using these tumor-associated antigen derived peptides. Unfortunately, only a low objective response rate has been observed in these cancer vaccine trials so far (NPL 11: Belli F et al., J Clin Oncol 2002 Oct 15, 20(20): 4169-80; NPL 12: Coulie PG et al., Immunol Rev 2002 Oct, 188: 33-42; NPL 13: Rosenberg SA et al., Nat Med 2004 Sep, 10(9): 909-15).

[0005] Recently, algorithms for predicting HLA class I-binding peptide sequences have been developed (NPL 14: Journal of Immunological Methods, (1995), Vol.185, pp.181-190, NPL 15: J. Immunol., (1994), Vol.152, pp.163-175, NPL 16: protein science, (2000), Vol.9, pp.1838-1846). However, it is difficult to estimate if a predicted epitope peptide can be processed naturally in the target cells and expressed on the target cell surface with HLA molecule. Moreover, the algorithms, for example BIMAS (bimas.dcrt.nih.gov/cgi-bin/molbio/ken_parker_comboform) (NPL 17: Parker KC, et al., (1994) J Immunol.;152(1):163-75.; NPL18: Kuzushima K, et al., (2001) Blood.;98(6):1872-81.) can suggest less than rigorous HLA molecule-binding peptides (NPL 19: Bachinsky MM, et al., Cancer Immun. 2005 Mar 22;5:6.). Thus TAA screening remains challenging and difficult.

[0006] Inhibins are heterodimeric glycoproteins composed of an alpha subunit (INHA) and one of two beta subunits (beta-A or beta-B). Inhibin, beta B (INHBB) is a subunit of both inhibin and activin, two closely related glycoproteins with opposing biological effects. Through gene expression profile analysis using a genome-wide cDNA microarray containing 23,040 genes, INHBB was recently shown to be up-regulated in several cancers such as non-small cell lung cancer (NSCLC), renal cell carcinoma (PTL 1: WO2005/019475; PTL 2: WO2007/013575) and esophageal cancer (See PTL 3: WO2004/031413, PTL 4: WO2005/019475, PTL 5: WO2007/013575, and PTL 6: WO2007/013671, the disclosures of which are incorporated by reference herein). Accordingly, INHBB is an interesting target for cancer immunotherapy and CTL inducing epitope peptides derived therefrom are sought by those in the art.

Citation List

Non Patent Literature

[0007] [NPL 1] Boon T, Int J Cancer 1993 May 8, 54(2): 177-80
[NPL 2] Boon T & van der Bruggen P, J Exp Med 1996 Mar 1, 183(3): 725-9
[NPL 3] Harris CC, J Natl Cancer Inst 1996 Oct 16, 88(20): 1442-55
[NPL 4] Butterfield LH et al., Cancer Res 1999 Jul 1, 59(13): 3134-42
[NPL 5] Vissers JL et al., Cancer Res 1999 Nov 1, 59(21): 5554-9
[NPL 6] van der Burg SH et al., J Immunol 1996 May 1, 156(9): 3308-14
[NPL 7] Tanaka F et al., Cancer Res 1997 Oct 15, 57(20): 4465-8
[NPL 8] Fujie T et al., Int J Cancer 1999 Jan 18, 80(2): 169-72
[NPL 9] Kikuchi M et al., Int J Cancer 1999 May 5, 81(3): 459-66
[NPL 10] Oiso M et al., Int J Cancer 1999 May 5, 81(3): 387-94
[NPL 11] Belli F et al., J Clin Oncol 2002 Oct 15, 20(20): 4169-80
[NPL 12] Coulie PG et al., Immunol Rev 2002 Oct, 188: 33-42
[NPL 13] Rosenberg SA et al., Nat Med 2004 Sep, 10(9): 909-15

[NPL 14] Journal of Immunological Methods, (1995), Vol.185, pp.181-190
[NPL 15] J. Immunol., (1994), Vol.152, pp.163-175
[NPL 16] protein science, (2000), Vol.9, pp.1838-1846
[NPL 17] Parker KC, et al., (1994) J Immunol.;152(1):163-75.
[NPL 18] Kuzushima K, et al., (2001) Blood.;98(6):1872-81.
[NPL 19] Bachinsky MM, et al., Cancer Immun. 2005 Mar 22;5:6.

Patent Literature

[0008] [PTL 1] WO2005/019475
[PTL 2] WO2007/013575
[PTL 3] WO2004/031413
[PTL 4] WO2005/019475
[PTL 5] WO2007/013575
[PTL 6] WO2007/013671

Summary of Invention

[0009] The present invention is based in part on the discovery of suitable targets of immunotherapy. Because TAAs often induce immune tolerance and therefore elicit poor immunogenicity, the discovery of appropriate targets is of extreme importance. Recognizing that INHBB has been identified as up-regulated in cancer tissues of cholangiocellular carcinoma, esophageal cancer, non-small cell lung cancer (NSCLC), renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor, the present invention targets the Homo sapiens inhibin, beta B (INHBB) protein (SEQ ID NO: 16) encoded by the gene of GenBank Accession No. NM_002193 (SEQ ID NO: 15)) for further analysis. In particular, INHBB gene products containing epitope peptides that elicit CTLs specific to the corresponding molecules were selected for study. Peripheral blood mononuclear cells (PBMCs) obtained from a healthy donor were stimulated using HLA-A*0201 binding candidate peptides derived from INHBB. CTLs that specifically recognize HLA-A02 positive target cells pulsed with the respective candidate peptides were established, and HLA-A02 restricted epitope peptides that can induce potent and specific immune responses against INHBB expressed on the surface of tumor cells were identified. These results demonstrate that INHBB is strongly immunogenic and the epitopes thereof are effective targets for tumor immunotherapy.

Accordingly, it is an object of the present invention to provide peptides having CTL inducibility as well as an amino acid sequence selected from the group of SEQ ID NOs: 1 to 14, and which have CTL inducibility. In addition, the present invention contemplates modified peptides, wherein one, two or more amino acids are substituted or added, so long as the modified peptides retain the original CTL inducibility.

When administered to a subject, the present peptides are presented on the surface of

antigen-expressing cells and then induce CTLs targeting the respective peptides. Therefore, it is an object of the present invention to provide antigen-presenting cells and exosomes that present any of the present peptides, as well as methods for inducing antigen-presenting cells.

[0010] An anti-tumor immune response is induced by the administration of the present INHBB polypeptides or polynucleotide encoding the polypeptides, as well as exosomes and antigen-presenting cells which present the INHBB polypeptides. Therefore, it is yet another object of the present invention to provide pharmaceutical agents containing the polypeptides or polynucleotides encoding them, as well as the exosomes and antigen-presenting cells as their active ingredients. The pharmaceutical agents of the present invention find use as vaccines.

[0011] Moreover, it is a further object of the present invention to provide methods for the treatment and/or prophylaxis of (i.e., prevention) cancers (tumors), and/or prevention of postoperative recurrence thereof, as well as methods for inducing CTLs, methods for inducing an immune response against tumor-associated endothelia and also anti-tumor immunity, which methods include the step of administering the INHBB polypeptides, polynucleotides encoding INHBB polypeptides, exosomes or the antigen-presenting cells presenting INHBB polypeptides or the pharmaceutical agents of the invention. In addition, the CTLs of the invention also find use as vaccines against cancer. Examples of the cancer include, but are not limited to, cholangio cellular carcinoma, esophageal cancer, non-small cell lung cancer (NSCLC), renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor.

[0012] It is to be understood that both the foregoing summary of the invention and the following detailed description are of exemplified embodiments, and not restrictive of the invention or other alternate embodiments of the invention.

Brief Description of Drawings

[0013] Various aspects and applications of the present invention will become apparent to the skilled artisan upon consideration of the brief description of the figures and the detailed description of the present invention and its preferred embodiments which follows:

[0014] [fig.1]Figure 1 includes a series of photographs, (a) - (n), depicting the results of IFN-gamma ELISPOT assay on CTLs that were induced with peptides derived from INHBB. The CTLs in well #4 stimulated with INHBB-A02-9-213 (SEQ ID NO: 1) (a), well #5 and #7 stimulated with INHBB-A02-9-174 (SEQ ID NO: 2) (b), well #8 stimulated with INHBB-A02-9-257 (SEQ ID NO: 3) (c), well #1 and #8 stimulated with INHBB-A02-9-313 (SEQ ID NO: 4) (d), well #1, #4 and #8 stimulated with INHBB-A02-9-139 (SEQ ID NO: 5) (e), well #4 stimulated with INHBB-A02-9-8

(SEQ ID NO: 6) (f), well #6 stimulated with INHBB-A02-9-250 (SEQ ID NO: 7) (g), well #5 stimulated with INHBB-A02-10-179 (SEQ ID NO: 8) (h), well #3 stimulated with INHBB-A02-10-237 (SEQ ID NO: 9) (i), well #5 stimulated with INHBB-A02-10-313 (SEQ ID NO: 10) (j), well #3 and #7 stimulated with INHBB-A02-10-173 (SEQ ID NO: 11) (k), well #4 stimulated with INHBB-A02-10-256 (SEQ ID NO: 12) (l), well #7 stimulated with INHBB-A02-10-162 (SEQ ID NO: 13) (m) and well #7 stimulated with INHBB-A02-10-85 (SEQ ID NO: 14) (n) showed potent IFN-gamma production as compared with the control respectively. In the figures, "+" indicates that the target cells in the well were pulsed with the appropriate peptide, and "-" indicates that the target cells had not been pulsed with any peptides.

[0015] [fig.2]Figure 2 depicts a line graph showing the results of establishment of CTL lines stimulated with INHBB-A02-9-174 (SEQ ID NO: 2) with IFN-gamma ELISA assay. The depicted results demonstrate that CTL line established by stimulation with the peptide showed potent IFN-gamma production as compared with the control. In the figures, "+" indicates that the target cells were pulsed with the appropriate peptide and "-" indicates that the target cells had not been pulsed with any peptides.

Description of Embodiments

[0016] 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.

[0017] The disclosure of each publication, patent or patent application mentioned in this specification is specifically incorporated by reference herein in its entirety. However, nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0018] In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0019] I. Definitions

The words "a", "an", and "the" as used herein mean "at least one" unless otherwise specifically indicated.

[0020] The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is a modified residue, or a non-naturally occurring residue, such as an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

[0021] The term "amino acid" as used herein refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that similarly function 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). 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). The phrase "amino acid mimetic" refers to chemical compounds that have different structures but similar functions to general amino acids.

[0022] Amino acids may be referred to herein by their commonly known three letter symbols or the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

[0023] The terms "gene", "polynucleotides", "nucleotides" and "nucleic acids" are used interchangeably herein unless otherwise specifically indicated and are similarly to the amino acids referred to by their commonly accepted single-letter codes.

[0024] Unless otherwise defined, the terms "cancer" refers to cancers over-expressing the INHBB gene, examples of which include, but are not limited to, cholangio cellular carcinoma, esophageal cancer, non-small cell lung cancer (NSCLC), renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor.

[0025] Unless otherwise defined, the terms "cytotoxic T lymphocyte", "cytotoxic T cell" and "CTL" are used interchangeably herein and, unless otherwise specifically indicated, refer to a sub-group of T lymphocytes that are capable of recognizing non-self cells (e.g., tumor cells, virus-infected cells) and inducing the death of such cells.

[0026] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0027] II. Peptides

To demonstrate that peptides derived from INHBB function as an antigen recognized by cytotoxic T lymphocytes (CTLs), peptides derived from INHBB (SEQ ID NO: 16) were analyzed to determine whether they were antigen epitopes restricted by HLA-A02 which are commonly encountered HLA alleles (Date Y et al., Tissue Antigens 47:

93-101, 1996; Kondo A et al., J Immunol 155: 4307-12, 1995; Kubo RT et al., J Immunol 152: 3913-24, 1994). Candidates of HLA-A02 binding peptides derived from INHBB were identified based on their binding affinities to HLA-A02. After in vitro stimulation of T-cells by dendritic cells (DCs) loaded with these peptides, CTLs were successfully established using each of the peptides of SEQ ID NOs: 1 to 14, particularly following peptides.

INHBB-A02-9-213 (SEQ ID NO: 1),
INHBB-A02-9-174 (SEQ ID NO: 2),
INHBB-A02-9-257 (SEQ ID NO: 3),
INHBB-A02-9-313 (SEQ ID NO: 4),
INHBB-A02-9-139 (SEQ ID NO: 5),
INHBB-A02-9-8 (SEQ ID NO: 6),
INHBB-A02-9-250 (SEQ ID NO: 7),
INHBB-A02-10-179 (SEQ ID NO: 8),
INHBB-A02-10-237 (SEQ ID NO: 9),
INHBB-A02-10-313 (SEQ ID NO: 10),
INHBB-A02-10-173 (SEQ ID NO: 11),
INHBB-A02-10-256 (SEQ ID NO: 12),
INHBB-A02-10-162 (SEQ ID NO: 13)
and
INHBB-A02-10-85 (SEQ ID NO: 14).

[0028] These established CTLs show potent specific CTL activity against target cells pulsed with respective peptides. The results herein demonstrate that INHBB is an antigen recognized by CTL and that the peptides may be epitope peptides of INHBB restricted by HLA-A02.

[0029] Since the INHBB gene is over expressed in most cancer tissues, such as cholangio cellular carcinoma, esophageal cancer, non-small cell lung cancer (NSCLC), renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor, it is a good target for immunotherapy. Thus, the present invention provides nonapeptides (peptides consisting of nine amino acid residues) and decapeptides (peptides consisting of ten amino acid residues) corresponding to CTL-recognized epitopes of INHBB. Particularly preferred examples of nonapeptides and decapeptides of the present invention include those peptides having an amino acid sequence selected from among SEQ ID NOs: 1 to 14.

[0030] Generally, software programs presently available on the Internet, such as those described in Parker KC et al., J Immunol 1994 Jan 1, 152(1): 163-75, can be used to calculate the binding affinities between various peptides and HLA antigens in silico. Binding affinity with HLA antigens can be measured as described, for example, in the

references Parker KC et al., J Immunol 1994 Jan 1, 152(1): 163-75; and Kuzushima K et al., Blood 2001, 98(6): 1872-81. The methods for determining binding affinity are described, for example, in; Journal of Immunological Methods, 1995, 185: 181-190; Protein Science, 2000, 9: 1838-1846. Thus, the present invention encompasses peptides of INHBB which bind with HLA antigens identified using such known programs.

[0031] The nonapeptides and decapeptides of the present invention can be flanked with additional amino acid residues so long as the resulting peptides retain their CTL inducibility. Such peptides having CTL inducibility are typically less than about 40 amino acids, often less than about 20 amino acids, usually less than about 15 amino acids. The particular amino acid sequences flanking the nonapeptides and decapeptides of the present invention (i.e., peptides consisting of the amino acid sequence selected from among SEQ ID NOs: 1 to 14) is not limited and can be composed of any kind of amino acids so long as it does not impair the CTL inducibility of the original peptide. Thus, the present invention also provides peptides having CTL inducibility and the amino acid sequence selected from among SEQ ID NOs: 1 to 14.

[0032] In general, modification of one, two, or more amino acids in a protein will not influence the function of the protein, and, in some cases will even enhance the desired function of the original protein. In fact, modified peptides (i.e., peptides composed of an amino acid sequence in which one, two or several amino acid residues have been modified (i.e., substituted, deleted, added or inserted) as compared to an original reference sequence) have been 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 may have both CTL inducibility and an amino acid sequence selected from among SEQ ID NO: 1 to 14 wherein one, two or even more amino acids are deleted, inserted, added and/or substituted.

[0033] Those of skill in the art recognize that individual additions or substitutions to an amino acid sequence which alters 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. As such, they are conventionally referred to as "conservative substitutions" or "conservative modifications", wherein the alteration of a protein results in a modified protein having properties and functions analogous to the original protein. Conservative substitution tables providing functionally similar amino acids are well known in the art. Examples of amino acid side chain characteristics that are desirable to conserve 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:

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

[0034] Such conservatively modified peptides are also considered to be 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 inducibility of the original peptide. Furthermore, modified peptides should not exclude CTL inducible peptides of polymorphic variants, interspecies homologues, and alleles of INHBB.

[0035] To retain the requisite CTL inducibility one can modify (insert, delete, add and/or substitute) 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, 3 or fewer. The percentage of amino acids to be modified is preferably 20% or less, more preferably 15% of less, even more preferably 10% or less or 1 to 5%.

[0036] Homology analysis of preferred peptides of the present invention, INHBB-A02-9-213 (SEQ ID NO: 1), INHBB-A02-9-174 (SEQ ID NO: 2), INHBB-A02-9-257 (SEQ ID NO: 3), INHBB-A02-9-313 (SEQ ID NO: 4), INHBB-A02-9-139 (SEQ ID NO: 5), INHBB-A02-9-8 (SEQ ID NO: 6), INHBB-A02-9-250 (SEQ ID NO: 7), INHBB-A02-10-179 (SEQ ID NO: 8), INHBB-A02-10-237 (SEQ ID NO: 9), INHBB-A02-10-313 (SEQ ID NO: 10), INHBB-A02-10-173 (SEQ ID NO: 11), INHBB-A02-10-256 (SEQ ID NO: 12), INHBB-A02-10-162 (SEQ ID NO: 13) and INHBB-A02-10-85 (SEQ ID NO: 14) confirmed that these peptides do not have significant homology with peptides derived from any other known human gene products. Thus, the possibility of these peptides generating unknown or undesired immune responses when used for immunotherapy is significantly lowered. Accordingly, these peptides are expected to be highly useful for eliciting immunity in tumor patients against INHBB on cancer cells, such as cholangio cellular carcinoma, esophageal cancer, non-small

cell lung cancer (NSCLC), renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor.

[0037] When used in the context of immunotherapy, peptides of the present invention should be presented on the surface of a cell or exosome, preferably as a complex with an HLA antigen. Therefore, it is preferable to select peptides that not only induce CTLs but also that possess high binding affinity to the HLA antigen. To that end, the peptides can be modified by substitution, insertion, deletion, and/or addition of the amino acid residues to yield a modified peptide having improved binding affinity. In addition to peptides that are naturally displayed, since the regularity of the sequences of peptides displayed by binding to HLA antigens is already known (J Immunol 1994, 152: 3913; Immunogenetics 1995, 41: 178; J Immunol 1994, 155: 4307), modifications based on such regularity can be introduced into the immunogenic peptides of the invention. For example, it may be desirable to substitute the second amino acid from the N-terminus substituted with leucine or methionine, and/or the amino acid at C-terminus with valine or leucine in order to increase the HLA-A0201 binding. Thus, peptides having the amino acid sequences of SEQ ID NOs: 1 to 14 wherein the second amino acid from the N-terminus of the amino acid sequence of the SEQ ID NOs is substituted with leucine or methionine and/or wherein the C-terminus of the amino acid sequence of the SEQ ID NOs is substituted with valine or leucine, are encompassed by the present invention. Substitutions can be introduced not only at the terminal amino acids but also at the position of potential TCR recognition of peptides. Several studies have demonstrated that amino acid substitutions in a peptide can be equal to or better than the original, for example, CAP1, $p53_{(264\text{-}272)}$, $Her\text{-}2/neu_{(369\text{-}377)}$ or $gp100_{(209\text{-}217)}$ (Zaremba et al., Cancer Res. 57, 4570-4577, 1997, T. K. Hoffmann et al., J Immunol. (2002) Feb 1;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, 307-314).

[0038] The present invention also contemplates the addition one to two amino acids to the N and/or C-terminus of the present peptides. Such modified peptides having high HLA antigen binding affinity and retained CTL inducibility are also included in the present invention.

[0039] However, when the peptide sequence 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 first perform homology searches using available databases to avoid situations in which the sequence of the peptide matches the amino acid sequence of another protein. When it becomes clear from the homology searches that there exists not even a peptide with 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 inducibility without any danger of such side effects.

[0040] Although peptides having high binding affinity to the HLA antigens as described above are expected to be highly effective, the candidate peptides, which are selected according to the presence of high binding affinity as an indicator, are further examined for the presence of CTL inducibility. Herein, the phrase "CTL inducibility" indicates the ability of the peptide to induce cytotoxic lymphocytes (CTLs) when presented on antigen-presenting cells. Further, "CTL inducibility" includes the ability of the peptide to induce CTL activation and/or CTL proliferation, promote CTL lysis of target cells, and to increase CTL IFN-gamma production.

[0041] Confirmation of CTL inducibility is accomplished by inducing antigen-presenting cells carrying human MHC antigens (for example, B-lymphocytes, macrophages, and dendritic cells (DCs)), or more specifically DCs derived from human peripheral blood mononuclear leukocytes, and after stimulation with the peptides, mixing with CD8-positive cells, and then measuring the IFN-gamma produced and released by CTL against the target cells. As the reaction system, transgenic animals that have been produced to express a human HLA antigen (for example, those described in BenMohamed L, Krishnan R, Longmate J, Auge C, Low L, Primus J, Diamond DJ, Hum Immunol 2000 Aug, 61(8): 764-79, Related Articles, Books, Linkout Induction of CTL response by a minimal epitope vaccine in HLA A*0201/DR1 transgenic mice: dependence on HLA class II restricted T(H) response) can be used. For example, the target cells can be radiolabeled with ^{51}Cr and such, and cytotoxic activity can be calculated from radioactivity released from the target cells. Alternatively, CTL inducibility can be assessed by measuring IFN-gamma produced and released by CTL in the presence of antigen-presenting cells (APCs) that carry immobilized peptides, and visualizing the inhibition zone on the media using anti-IFN-gamma monoclonal antibodies.

[0042] As a result of examining the CTL inducibility of the peptides as described above, it was discovered that those peptides having high binding affinity to an HLA antigen did not necessarily have high CTL inducibility. However, of those peptides identified and assessed, nonapeptides or decapeptides selected from peptides having an amino acid sequence selected from among SEQ ID NOs: 1 to 14 were found to exhibit particularly high CTL inducibility as well as high binding affinity to an HLA antigen. Thus, these peptides are exemplified as preferred embodiments of the present invention.

[0043] In addition to the above-described modifications, the peptides of the present invention can also be linked to other substances, so long as the resulting linked peptide retains the requisite CTL inducibility of the original peptide. Examples of suitable

substances include, but are not limited to: peptides, lipids, sugar and sugar chains, acetyl groups, natural and synthetic polymers, etc. The peptides can contain modifications such as glycosylation, side chain oxidation, or phosphorylation, etc., provided the modifications do not destroy the biological activity of the original peptide. These kinds of modifications can be performed to confer additional functions (e.g., targeting function, and delivery function) or to stabilize the polypeptide.

[0044] For example, to increase the in vivo stability of a polypeptide, it is known in the art to introduce D-amino acids, amino acid mimetics or unnatural amino acids; this concept can also be adapted to the present polypeptides. The stability of a polypeptide can be assayed in a number of ways. For instance, peptidases and various biological media, such as human plasma and serum, can be used to test stability (see, e.g., Verhoef et al., Eur J Drug Metab Pharmacokin 1986, 11: 291-302).

[0045] Further, the peptides of the present invention may be linked to other peptides via spacers or linkers. Examples of other peptides include, but are not limited to, CTL inducible peptides derived from other TAAs. Alternatively, two or more peptides of the present invention may be linked via spacers or linkers. The peptides linked via spacers or linkers may be the same or different each other. Spacers or linkers are not specifically limited, but are preferably peptides, more preferably peptides having one or more cleavage sites which are capable of being cleaved by enzymes such as peptidases, proteases and proteasomes. Examples of linkers or spacers include, but are not limited to: AAY (P. M. Daftarian et al., J Trans Med 2007, 5:26), AAA, NKRK (R. P. M. Sutmuller et al., J Immunol. 2000, 165: 7308-7315) or, one to several lysine redsidues (S. Ota et al., Can Res. 62, 1471-1476, K. S. Kawamura et al., J Immunol. 2002, 168: 5709-5715). The peptide of the present invention encompass those peptides linked to other peptides via spacers or linkers.

[0046] The peptides of the present invention may be existed on the surface of a cell carrying human MHC antigens (e.g. antigen presenting cell) or an exosome as complexes in combination with MHC molecules and then induce CTLs. The cells and the exosomes can be prepared by well-known methods in the art, for example, the cells may be prepared by contacting with the peptides of the present invention, and the exosomes may be prepared by collecting an exosome-containing fraction from the cells contacted with the peptides of the present invention (see, e.g., Japanese Patent Application Kohyo Publications Nos. Hei 11-510507 and WO99/03499). The peptides of the present invention encompass those peptides existed on the surface of a cell or an exosome as complexes in combination with MHC molecules.

[0047] Herein, the peptides of the present invention can also be described as "INHBB peptide(s)" or "INHBB polypeptide(s)".

[0048] III. Preparation of INHBB peptides

The peptides of the present invention can be prepared using well known techniques. For example, the peptides can be prepared synthetically, using recombinant DNA technology or chemical synthesis. Peptides of the present invention can be synthesized individually or as longer polypeptides composed of two or more peptides. The peptides can then be isolated i.e., purified or isolated so as to be substantially free of other naturally occurring host cell proteins and fragments thereof, or any other chemical substances.

[0049] 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, but are not limited to:

(i) Peptide Synthesis, Interscience, New York, 1966;

(ii) The Proteins, Vol. 2, Academic Press, New York, 1976;

(iii) Peptide Synthesis (in Japanese), Maruzen Co., 1975;

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

(v) Development of Pharmaceuticals (second volume) (in Japanese), Vol. 14 (peptide synthesis), Hirokawa, 1991;

(vi) WO99/67288; and

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

[0050] Alternatively, the present peptides can be obtained adapting any known genetic engineering methods for producing peptides (e.g., Morrison J, J Bacteriology 1977, 132: 349-51; Clark-Curtiss & Curtiss, Methods in Enzymology (eds. Wu et al.) 1983, 101: 347-62). For example, first, a suitable vector harboring a polynucleotide encoding the objective peptide 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 interest. The peptide can also be produced in vitro adopting an in vitro translation system.

[0051] IV. Polynucleotides

The present invention also provides a polynucleotide which encodes any of the aforementioned peptides of the present invention. These include polynucleotides derived from the natural occurring INHBB gene (GenBank Accession No. NM_002193 (SEQ ID NO: 15)) 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

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.

[0052] 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.

[0053] 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 (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.

[0054] 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.

[0055] Vectors containing the polynucleotide of the present invention and host cells harboring the vectors are also included in the present invention.

[0056] V. Exosomes

The present invention further provides intracellular vesicles called exosomes, which present complexes formed between the peptides of the present invention and HLA antigens on their surface. Exosomes can be prepared, for example, by using the methods detailed in Japanese Patent Application Kohyo Publications Nos. Hei

11-510507 and WO99/03499, and can be prepared using APCs obtained from patients who are subject to treatment and/or prevention. The exosomes of this invention can be inoculated as vaccines, in a fashion similar to the peptides of this invention.

[0057] The type of HLA antigens included in the complexes must match that of the subject requiring treatment and/or prevention. For example, in the Japanese and Caucasian populations, HLA-A02 type is prevalent. Thus, the use of the A02 type is favorable for obtaining effective results in these populations, with subtypes such as A0201 also finding use. Typically, in the clinic, the type of HLA antigen of the patient requiring treatment is investigated in advance, which enables the appropriate selection of peptides having high levels of binding affinity to the particular antigen, or having CTL inducibility by antigen presentation. Furthermore, in order to obtain peptides having both high binding affinity and CTL inducibility, substitution, insertion and/or addition of 1, 2, or several amino acids can be performed based on the amino acid sequence of the naturally occurring INHBB partial peptide.

[0058] When using A02 type HLA antigen for the exosome of the present invention, the peptides having the sequence selected from among SEQ ID NO: 1 to 14 find use.

[0059] VI. Antigen-presenting cells (APCs)

The present invention also provides isolated APCs that present complexes formed between HLA antigens and the peptides of this invention on its surface. The APCs that are obtained by contacting the peptides of this invention, or introducing the nucleotides encoding the peptides of this invention in an expressible form can be derived from patients who are subject to treatment and/or prevention, and can be administered as vaccines by themselves or in combination with other drugs including the peptides of this invention, exosomes, or cytotoxic T cells.

[0060] The APCs are not limited to a particular kind of cells and include dendritic cells (DCs), Langerhans cells, macrophages, B cells, and activated T cells, which are known to present proteinaceous antigens on their cell surface so as to be recognized by lymphocytes. Since DC is a representative APC having the strongest CTL inducing action among APCs, DCs find use as the APCs of the present invention.

[0061] For example, an APC can be obtained by inducing DCs from peripheral blood monocytes and then contacting (stimulating) them with the peptides of this invention in vitro, ex vivo or in vivo. When the peptides of this invention are administered to the subjects, APCs that present the peptides of this invention are induced in the body of the subject. The phrase "inducing APC" includes contacting (stimulating) a cell with the peptides of this invention, or nucleotides encoding the peptides of this invention to present complexes formed between HLA antigens and the peptides of this invention on cell's surface. Alternatively, after introducing the peptides of this invention to the APCs to allow the APCs to present the peptides, the APCs can be administered to the

subject as a vaccine. For example, the ex vivo administration can include the steps of:
a: collecting APCs from a first subject,
b: contacting with the APCs of step a, with the peptide and
c: administering the peptide-loaded APCs to a second subject.

[0062] The first subject and the second subject can be the same individual, or may be different individuals. Alternatively, according to the present invention, use of the peptides of the present invention for manufacturing a pharmaceutical composition inducing antigen-presenting cells is provided. In addition, the present invention provides a method or process for manufacturing a pharmaceutical composition inducing antigen-presenting cells, wherein the method includes the step of admixing or formulating the peptide of the present invention with a pharmaceutically acceptable carrier. Further, the present invention also provides the peptides of the present invention for inducing antigen-presenting cells. The APCs obtained by step b can be administered to the subject as a vaccine.

[0063] According to an aspect of the present invention, the APCs of the present invention have a high level of CTL inducibility. In the term of "high level of CTL inducibility", the high level is relative to the level of that by APCs contacted with no peptide or peptides which can not induce the CTL. Such APCs having a high level of CTL inducibility can be prepared by a method which includes the step of transferring genes containing polynucleotides that encode the peptides of this invention to APCs in vitro. The introduced genes can be in the form of DNAs or RNAs. Examples of methods for introduction include, without particular limitations, various methods conventionally performed in this field, such as lipofection, electroporation, and calcium phosphate method can be used. More specifically, it can be performed as described in Cancer Res 1996, 56: 5672-7; J Immunol 1998, 161: 5607-13; J Exp Med 1996, 184: 465-72; Published Japanese Translation of International Publication No. 2000-509281. By transferring the gene into APCs, the gene undergoes transcription, translation, and such in the cell, and then the obtained protein is processed by MHC Class I or Class II, and proceeds through a presentation pathway to present peptides.

[0064] VII. Cytotoxic T cells (CTLs)

A cytotoxic T cell induced against any of the peptides of the present invention strengthens the immune response targeting tumor-associated endothelia in vivo and thus can be used as vaccines, in a fashion similar to the peptides per se. Thus, the present invention also provides isolated cytotoxic T cells that are specifically induced or activated by any of the present peptides.

[0065] Such cytotoxic T cells can be obtained by (1) administering the peptide of the present invention to a subject, and then collecting cytotoxic T cells from the subject, or (2) contacting (stimulating) subject-derived APCs, and CD8-positive cells, or peripheral

blood mononuclear leukocytes in vitro with the peptides of the present invention and then isolating cytotoxic T cells.

[0066] The cytotoxic T cells, which have been induced by stimulation with APCs that present the peptides of this invention, can be derived from patients who are subject to treatment and/or prevention, and can be administered by themselves or in combination with other drugs including the peptides of this invention or exosomes for the purpose of regulating effects. The obtained cytotoxic T cells act specifically against target cells presenting the peptides of this invention, or for example, the same peptides used for induction. In the other word, the cytotoxic T cells can recognize (i.e., binding to) a complex formed between a HLA antigen and the peptide of the present invention on a target cell surface with the T cell receptor and then attack the target cell to induce the death of the target cell. The target cells can be cells that endogenously express INHBB, or cells that are transfected with the INHBB gene; and cells that present a peptide of this invention on the cell surface due to stimulation by the peptide can also serve as targets of activated CTL attack.

[0067] VIII. T cell receptor (TCR)

The present invention also provides a composition composed of a nucleic acid sequence encoding polypeptides that are capable of forming a subunit of a T cell receptor (TCR), and methods of using the same. The TCR subunits have the ability to form TCRs that confer specificity to T cells against tumor cells presenting INHBB. By using the known methods in the art, the nucleic acid sequence of alpha- and beta-chains of the TCR expressed in the CTL induced with one or more peptides of this invention can be identified (WO2007/032255 and Morgan et al., J Immunol, 171, 3288 (2003)). The derivative TCRs can bind to the INHBB peptide displaying on the target cells with high avidity, and optionally mediate efficient killing of target cells presenting the INHBB peptide in vivo and in vitro.

[0068] The nucleic acids sequence encoding the TCR subunits can be incorporated into suitable vectors e.g. retroviral vectors. These vectors are well known in the art. The nucleic acids or the vectors containing them usefully can be transferred into a T cell, for example, a T cell from a patient. Advantageously, the invention provides an off-the-shelf composition allowing rapid modification of a patient's own T cells (or those of another mammal) to rapidly and easily produce modified T cells having excellent cancer cell killing properties.

[0069] Also, the present invention provides CTLs which are prepared by transduction with the nucleic acids encoding the TCR subunit polypeptides that bind to the INHBB peptide e.g. SEQ ID NOs: 1 to 14 in the context of HLA-A02. The transduced CTLs are capable of homing to cancer cells in vivo, and can be expanded by well known culturing methods in vitro (e.g., Kawakami et al., J Immunol., 142, 3452-3461 (1989)).

The T cells of the invention can be used to form an immunogenic composition useful in treating or the prevention of cancer in a patient in need of therapy or protection (WO2006/031221).

[0070] IX. Pharmaceutical agents or composition

Prevention and prophylaxis include any activity which reduces the burden of mortality or morbidity from disease. Prevention and prophylaxis can occur "at primary, secondary and tertiary prevention levels." While primary prevention and prophylaxis avoid the development of a disease, secondary and tertiary levels of prevention and prophylaxis encompass activities aimed at the prevention and prophylaxis of the progression of a disease and the emergence of symptoms as well as reducing the negative impact of an already established disease by restoring function and reducing disease-related complications. Alternatively, prevention and prophylaxis include a wide range of prophylactic therapies aimed at alleviating the severity of the particular disorder, e.g. reducing the proliferation and metastasis of tumors.

[0071] Treating and/or for the prophylaxis of cancer or tumor and/or the prevention of post-operative recurrence thereof includes any of the following steps, such as surgical removal of cancer cells, inhibition of the growth of cancerous cells, involution or regression of a tumor, induction of remission and suppression of occurrence of cancer, tumor regression, and reduction or inhibition of metastasis. Effectively treating and/or the prophylaxis of cancer decreases mortality and improves the prognosis of individuals having cancer, decreases the levels of tumor markers in the blood, and alleviates detectable symptoms accompanying cancer. For example, reduction or improvement of symptoms constitutes effectively treating and/or the prophylaxis include 10%, 20%, 30% or more reduction, or stable disease.

[0072] Since INHBB expression is up-regulated in several cancers as compared with normal tissue, the peptides of this invention or polynucleotides encoding such peptides can be used for the treatment and/or for the prophylaxis of cancer, and/or prevention of post-operative recurrence thereof. Thus, the present invention provides a pharmaceutical agent or composition for treating and/or preventing cancer, and/or preventing the post-operative recurrence thereof, which includes one or more of the peptides of this invention, or polynucleotides encoding the peptides as an active ingredient. Alternatively, the present peptides can be expressed on the surface of any of the foregoing exosomes or cells, such as APCs for the use as pharmaceutical agents or compositions. In addition, the aforementioned cytotoxic T cells which target any of the peptides of the present invention can also be used as the active ingredient of the present pharmaceutical agents or compositions. In the context of the present invention, the phrase "targeting a peptide" refers to recognizing (i.e., binding to) a complex formed between a HLA antigen and a peptide on a target cell surface with the T cell receptor, and then

attacking the target cell to induce the death of the target cell.

[0073] In another embodiment, the present invention also provides the use of an active ingredient selected from among:

(a) a peptide of the present invention,

(b) a nucleic acid encoding such a peptide as disclosed herein in an expressible form,

(c) an APC of the present invention, and

(d) a cytotoxic T cells of the present invention

in manufacturing a pharmaceutical composition or agent for treating cancer.

Alternatively, the present invention further provides an active ingredient selected from among:

(a) a peptide of the present invention,

(b) a nucleic acid encoding such a peptide as disclosed herein in an expressible form,

(c) an APC of the present invention, and

(d) a cytotoxic T cells of the present invention

for use in treating cancer.

[0074] Alternatively, the present invention further provides a method or process for manufacturing a pharmaceutical composition or agent for treating cancer, wherein the method or process includes the step of formulating a pharmaceutically or physiologically acceptable carrier with an active ingredient selected from among:

(a) a peptide of the present invention,

(b) a nucleic acid encoding such a peptide as disclosed herein in an expressible form,

(c) an APC of the present invention, and

(d) a cytotoxic T cells of the present invention

as active ingredients.

[0075] In another embodiment, the present invention also provides a method or process for manufacturing a pharmaceutical composition or agent for treating cancer, wherein the method or process includes the step of admixing an active ingredient with a pharmaceutically or physiologically acceptable carrier, wherein the active ingredient is selected from among:

(a) a peptide of the present invention,

(b) a nucleic acid encoding such a peptide as disclosed herein in an expressible form,

(c) an APC of the present invention, and

(d) a cytotoxic T cells of the present invention.

[0076] Alternatively, the pharmaceutical composition or agent of the present invention may be used for either or both the prophylaxis of cancer and prevention of postoperative recurrence thereof.

[0077] The present pharmaceutical agents or compositions find use as a vaccine. In the context of the present invention, the phrase "vaccine" (also referred to as an "im-

munogenic composition") refers to a substance that has the function to induce anti-tumor immunity upon inoculation into animals.

[0078] The pharmaceutical agents or compositions of the present invention can be used to treat and/or prevent cancers, and/or prevention of postoperative recurrence thereof in subjects or patients including human and any other mammal including, but not limited to, mouse, rat, guinea-pig, rabbit, cat, dog, sheep, goat, pig, cattle, horse, monkey, baboon, and chimpanzee, particularly a commercially important animal or a domesticated animal.

[0079] According to the present invention, polypeptides having an amino acid sequence selected from among SEQ ID NOs: 1 to 14 or polypeptides having an amino acid sequence selected from among SEQ ID NOs: 1 to 14 have been found to be HLA-A02 restricted epitope peptides or candidates, respectively, that can induce potent and specific immune response. Therefore, the present pharmaceutical agents or compositions which include any of these polypeptides with the amino acid sequences selected from among SEQ ID NOs: 1 to 14 are particularly suited for the administration to subjects whose HLA antigen is HLA-A02. The same applies to pharmaceutical agents or compositions which include polynucleotides encoding any of these polypeptides.

[0080] Cancers to be treated by the pharmaceutical agents or compositions of the present invention are not limited and include all kinds of cancers wherein INHBB is involved, including, for example, cholangio cellular carcinoma, esophageal cancer, non-small cell lung cancer (NSCLC), renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor.

[0081] The present pharmaceutical agents or compositions can contain in addition to the aforementioned active ingredients, other peptides which have the ability to induce CTLs against cancerous cells, other polynucleotides encoding the other peptides, other cells that present the other peptides, or such. Herein, the other peptides that have the ability to induce CTLs against cancerous cells are exemplified by cancer specific antigens (e.g., identified TAAs), but are not limited thereto.

[0082] If needed, the pharmaceutical agents or compositions of the present invention can optionally include other therapeutic substances as an active ingredient, so long as the substance does not inhibit the antitumoral effect of the active ingredient, e.g., any of the present peptides. For example, formulations can include anti-inflammatory agents or compositions, pain killers, chemotherapeutics, and the like. In addition to including other therapeutic substances in the medicament itself, the medicaments of the present invention can also be administered sequentially or concurrently with the one or more other pharmacologic agents or compositions. The amounts of medicament and pharmacologic agent or composition depend, for example, on what type of pharmacologic

agent(s) or composition(s) is/are used, the disease being treated, and the scheduling and routes of administration.

[0083] It should be understood that in addition to the ingredients particularly mentioned herein, the pharmaceutical agents or compositions of this invention can include other agents or compositions conventional in the art having regard to the type of formulation in question.

[0084] In one embodiment of the present invention, the present pharmaceutical agents or compositions can be included in articles of manufacture and kits containing materials useful for treating the pathological conditions of the disease to be treated, e.g., cancer. The article of manufacture can include a container of any of the present pharmaceutical agents or compositions with a label. Suitable containers include bottles, vials, and test tubes. The containers can be formed from a variety of materials, such as glass or plastic. The label on the container should indicate the agent or compositions is used for treating or prevention of one or more conditions of the disease. The label can also indicate directions for administration and so on.

[0085] In addition to the container described above, a kit including a pharmaceutical agent or compositions of the present invention can optionally further include a second container housing a pharmaceutically-acceptable diluent. It can further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[0086] The pharmaceutical compositions can, if desired, be presented in a pack or dispenser device which can contain one or more unit dosage forms containing the active ingredient. The pack can, for example, include metal or plastic foil, such as a blister pack. The pack or dispenser device can be accompanied by instructions for administration.

[0087] (1) Pharmaceutical agents or compositions containing the peptides as the active ingredient

The peptides of this invention can be administered directly as a pharmaceutical agent or composition, or if necessary, that has been formulated by conventional formulation methods. In the latter case, in addition to the peptides of this invention, carriers, excipients, and such that are ordinarily used for drugs can be included as appropriate without particular limitations. Examples of such carriers are sterilized water, physiological saline, phosphate buffer, culture fluid and such. Furthermore, the pharmaceutical agents or compositions can contain as necessary, stabilizers, suspensions, preservatives, surfactants and such. The pharmaceutical agents or compositions of this invention can be used for anticancer purposes.

[0088] The peptides of this invention can be prepared as a combination composed of two or more of peptides of the invention, to induce CTL in vivo. The peptide combination can

take the form of a cocktail or can be conjugated to each other using standard techniques. For example, the peptides can be chemically linked or expressed as a single fusion polypeptide sequence. The peptides in the combination can be the same or different. By administering the peptides of this invention, the peptides are presented at a high density by the HLA antigens on APCs, then CTLs that specifically react toward the complex formed between the displayed peptide and the HLA antigen are induced. Alternatively, APCs that present any of the peptides of this invention on their cell surface, which may be obtained by stimulating APCs (e.g., DCs) derived from a subject with the peptides of this invention, may be administered to the subject, and as a result, CTLs are induced in the subject and aggressiveness towards the cancer cells, such as cholangio cellular carcinoma, esophageal cancer, non-small cell lung cancer (NSCLC), renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor can be increased.

[0089] The pharmaceutical agents or compositions for the treatment and/or prevention of cancer, which include a peptide of this invention as the active ingredient, can also include an adjuvant known to effectively establish cellular immunity. Alternatively, they can be administered with other active ingredients, and they can be administered by formulation into granules. An adjuvant refers to a compound that enhances the immune response against the protein when administered together (or successively) with the protein having immunological activity. Adjuvants contemplated herein include those described in the literature (Clin Microbiol Rev 1994, 7: 277-89). Examples of suitable adjuvants include, but are not limited to, aluminum phosphate, aluminum hydroxide, alum, cholera toxin, salmonella toxin, and such, but are not limited thereto.

[0090] Furthermore, liposome formulations, granular formulations in which the peptide is bound to few-micrometers diameter beads, and formulations in which a lipid is bound to the peptide may be conveniently used.

[0091] In some embodiments, the pharmaceutical agents or compositions of the invention may further include a component which primes CTL. Lipids have been identified as agents or compositions capable of priming CTL 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 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-glycerylcysteinlyseryl- serine (P3CSS) can be used to prime CTL when covalently attached to an appropriate peptide (see, e.g., Deres et al., Nature 1989, 342: 561-4).

[0092] The method of administration can be oral, intradermal, subcutaneous, intravenous

injection, or such, and systemic administration or local administration to the vicinity of the targeted sites. The administration can be performed by single administration or boosted by multiple administrations. The dose of the peptides of this invention can be adjusted appropriately according to the disease to be treated, age of the patient, weight, method of administration, and such, and is ordinarily 0.001 mg to 1000 mg, for example, 0.001 mg to 1000 mg, for example, 0.1 mg to 10 mg, and can be administered once in a few days to few months. One skilled in the art can appropriately select a suitable dose.

[0093] (2) Pharmaceutical agents or compositions containing polynucleotides as the active ingredient

The pharmaceutical agents or compositions of the invention can also contain nucleic acids encoding the peptides disclosed herein in an expressible form. Herein, the phrase "in an expressible form" means that the polynucleotide, when introduced into a cell, will be expressed in vivo as a polypeptide that induces anti-tumor immunity. In an exemplified embodiment, the nucleic acid sequence of the polynucleotide of interest includes regulatory elements necessary for expression of the polynucleotide. The polynucleotide(s) can be equipped so to achieve stable insertion into the genome of the target cell (see, e.g., Thomas KR & Capecchi MR, 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. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; and WO 98/04720. 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. Patent No. 5,922,687).

[0094] 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. This approach involves the use of vaccinia virus, e.g., as a vector to express nucleotide sequences that encode the peptide. 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. Patent No. 4,722,848. Examples of another vector include 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.

[0095] Delivery of a polynucleotide into a subject can be either direct, in which case the

subject is directly exposed to a polynucleotide-carrying vector, or indirect, in which case, cells are first transformed with the polynucleotide of interest in vitro, then the cells are transplanted into the subject. Theses two approaches are known, respectively, as in vivo and ex vivo gene therapies.

[0096] 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 eds. 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.

[0097] The method of administration can be oral, intradermal, subcutaneous, intravenous injection, or such, and systemic administration or local administration to the vicinity of the targeted sites finds use. The administration can be performed by single administration or boosted by multiple administrations. The dose of the polynucleotide in the suitable carrier or cells transformed with the polynucleotide encoding the peptides of this invention can be adjusted appropriately according to the disease to be treated, age of the patient, weight, method of administration, and such, and is ordinarily 0.001 mg to 1000 mg, for example, 0.001 mg to 1000 mg, for example, 0.1 mg to 10 mg, and can be administered once every a few days to once every few months. One skilled in the art can appropriately select the suitable dose.

[0098] X. Methods using the peptides, exosomes, APCs and CTLs

The peptides of the present invention and polynucleotides encoding such peptides can be used for inducing APCs and CTLs. The exosomes and APCs of the present invention can be also used for inducing CTLs. The peptides, polynucleotides, exosomes and APCs can be used in combination with any other compounds so long as the compounds do not inhibit their CTL inducibility. Thus, any of the aforementioned pharmaceutical agents or compositions of the present invention can be used for inducing CTLs, and in addition thereto, those including the peptides and polynucleotides can be also be used for inducing APCs as discussed below.

[0099] (1) Method of inducing antigen-presenting cells (APCs)

The present invention provides methods of inducing APCs using the peptides of this invention or polynucleotides encoding the peptides. The induction of APCs can be performed as described above in section "VI. Antigen-presenting cells". This invention also provides a method for inducing APCs having a high level of CTL inducibility, the induction of which has been also mentioned under the item of "VI. Antigen-presenting

cells", supra.

Preferably, the methods for inducing APCs include at least one step selected from among:

a: contacting APCs with the peptides of the present invention, and

b: introducing the polypeptides of the present invention in an expressible form into APCs.

Such methods for inducing APCs are preferably performed in vitro or ex vivo. When the methods performed in vitro or ex vivo, APCs to be induced may be obtained from a subject to be treated or others whose HLA antigens are the same as the subject.

[0100] (2) Method of inducing CTLs

Furthermore, the present invention provides methods for inducing CTLs using the peptides of this invention, polynucleotides encoding the peptides, or exosomes or APCs presenting the peptides.

[0101] The present invention also provides methods for inducing CTLs using a polynucleotide encoding a polypeptide that is capable of forming a T cell receptor (TCR) subunit recognizing (i.e., binding to) a complex of the peptides of the present invention and HLA antigens on a cell surface. Preferably, the methods for inducing CTLs include at least one step selected from among:

a: contacting a CD8-positive T cell with an antigen-presenting cell and/or an exosome that presents on its surface a complex of an HLA antigen and a peptide of the present invention, and

b: introducing a polynucleotide encoding a polypeptide that is capable of forming a TCR subunit recognizing a complex of a peptide of the present invention and an HLA antigen into a CD8 positive T cell.

[0102] When the peptides of this invention are administered to a subject, CTL is induced in the body of the subject, and the strength of the immune response targeting the tumor-associated endothelia is enhanced. Alternatively, the peptides and polynucleotides encoding the peptides can be used for an ex vivo therapeutic method, in which subject-derived APCs, and CD8-positive cells, or peripheral blood mononuclear leukocytes are contacted (stimulated) with the peptides of this invention in vitro, and after inducing CTL, the activated CTL cells are returned to the subject. For example, the method can include steps of:

a: collecting APCs from subject,

b: contacting with the APCs of step a, with the peptide,

c: mixing the APCs of step b with CD^{8+} T cells, and co-culturing for inducing CTLs, and

d: collecting CD^{8+} T cells from the co-culture of step c.

[0103] Alternatively, according to the present invention, use of the peptides of this invention

for manufacturing a pharmaceutical composition inducing CTLs is provided. In addition, the present invention provides a method or process for manufacturing a pharmaceutical agent or composition inducing CTLs, wherein the method includes the step of admixing or formulating the peptide of the present invention with a pharmaceutically acceptable carrier. Further, the present invention also provides the peptide of the present invention for inducing CTLs.

[0104] The CD^{8+} T cells having cytotoxic activity obtained by step d can be administered to the subject as a vaccine. The APCs to be mixed with the CD^{8+} T cells in above step c can also be prepared by transferring genes coding for the present peptides into the APCs as detailed above in section "VI. Antigen-presenting cells"; but are not limited thereto and any APC or exosome which effectively presents the present peptides to the T cells can be used for the present method.

[0105] The following examples are presented to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention.

Examples

[0106] Materials and Methods

Cell lines

H2 (HLA-A02), human B-lymphoblastoid cell line, and COS7 were purchased from ATCC.

[0107] Candidate selection of peptides derived from INHBB

9-mer and 10-mer peptides derived from INHBB that bind to HLA-A*0201 molecules were predicted using binding prediction software "BIMAS" (www-bimas.cit.nih.gov/molbio/hla_bind), which algorithms had been described by Parker KC et al.(J Immunol 1994, 152(1): 163-75) and Kuzushima K et al.(Blood 2001, 98(6): 1872-81). These peptides were synthesized by Sigma (Sapporo, Japan) or Biosynthesis Inc. (Lewisville, TX) according to a standard solid phase synthesis method and purified by reversed phase high performance liquid chromatography (HPLC). The purity (>90%) and the identity of the peptides were determined by analytical HPLC and mass spectrometry analysis, respectively. Peptides were dissolved in dimethylsulfoxide (DMSO) at 20 mg/ml and stored at -80 degrees C.

[0108] In vitro CTL Induction

Monocyte-derived dendritic cells (DCs) were used as antigen-presenting cells (APCs) to induce cytotoxic T lymphocyte (CTL) responses against peptides presented on human leukocyte antigen (HLA). DCs were generated in vitro as described elsewhere (Nakahara S et al., Cancer Res 2003 Jul 15, 63(14): 4112-8). Specifically, peripheral blood mononuclear cells (PBMCs) isolated from a normal volunteer

(HLA-A*0201 positive) by Ficoll-Plaque (Pharmacia) solution were separated by adherence to a plastic tissue culture dish (Becton Dickinson) so as to enrich them as the monocyte fraction. The monocyte-enriched population was cultured in the presence of 1000 U/ml of granulocyte-macrophage colony-stimulating factor (GM-CSF) (R&D System) and 1000 U/ml of interleukin (IL)-4 (R&D System) in AIM-V Medium (Invitrogen) containing 2% heat-inactivated autologous serum (AS). After 7 days of culture, the cytokine-induced DCs were pulsed with 20 mcg/ml of each of the synthesized peptides in the presence of 3 mcg/ml of beta2-microglobulin for 3 hr at 37 degrees C in AIM-V Medium. The generated cells appeared to express DC-associated molecules, such as CD80, CD83, CD86 and HLA class II, on their cell surfaces (data not shown). These peptide-pulsed DCs were then inactivated by Mitomycin C (MMC) (30 mcg/ml for 30 min) and mixed at a 1:20 ratio with autologous CD8+ T cells, obtained by positive selection with CD8 Positive Isolation Kit (Dynal). These cultures were set up in 48-well plates (Corning); each well contained 1.5 x 10^4 peptide-pulsed DCs, 3 x 10^5 CD8+ T cells and 10 ng/ml of IL-7 (R&D System) in 0.5 ml of AIM-V/2% AS medium. Three days later, these cultures were supplemented with IL-2 (CHIRON) to a final concentration of 20 IU/ml. On day 7 and 14, the T cells were further stimulated with the autologous peptide-pulsed DCs. The DCs were prepared each time by the same way described above. CTL was tested against peptide-pulsed T2 cells after the 3rd round of peptide stimulation on day 21 (Tanaka H et al., Br J Cancer 2001 Jan 5, 84(1): 94-9; Umano Y et al., Br J Cancer 2001 Apr 20, 84(8): 1052-7; Uchida N et al., Clin Cancer Res 2004 Dec 15, 10(24): 8577-86; Suda T et al., Cancer Sci 2006 May, 97(5): 411-9; Watanabe T et al., Cancer Sci 2005 Aug, 96(8): 498-506).

[0109] CTL Expansion Procedure

CTLs were expanded in culture using the method similar to the one described by Riddell et al. (Walter EA et al., N Engl J Med 1995 Oct 19, 333(16): 1038-44; Riddell SR et al., Nat Med 1996 Feb, 2(2): 216-23). A total of 5 x 10^4 CTLs were suspended in 25 ml of AIM-V/5% AS medium with 2 kinds of human B-lymphoblastoid cell lines, inactivated by MMC, in the presence of 40 ng/ml of anti-CD3 monoclonal antibody (Pharmingen). One day after initiating the cultures, 120 IU/ml of IL-2 were added to the cultures. The cultures were fed with fresh AIM-V/5% AS medium containing 30 IU/ml of IL-2 on days 5, 8 and 11 (Tanaka H et al., Br J Cancer 2001 Jan 5, 84(1): 94-9; Umano Y et al., Br J Cancer 2001 Apr 20, 84(8): 1052-7; Uchida N et al., Clin Cancer Res 2004 Dec 15, 10(24): 8577-86; Suda T et al., Cancer Sci 2006 May, 97(5): 411-9; Watanabe T et al., Cancer Sci 2005 Aug, 96(8): 498-506).

[0110] Specific CTL activity

To examine specific CTL activity, interferon (IFN)-gamma enzyme-linked immunospot (ELISPOT) assay and IFN-gamma enzyme-linked immunosorbent assay

(ELISA) were performed. Specifically, peptide-pulsed T2 (1 x 10^4/well) was prepared as stimulator cells. Cultured cells in 48 wells were used as responder cells. IFN-gamma ELISPOT assay and IFN-gamma ELISA assay were performed under manufacture procedure.

[0111] Results

Enhanced INHBB expression in cancers

INHBB expression was validly elevated in the following cancers: 10 out of 21 in cholangiocellular carcinoma, 12 out of 12 in esophageal cancer, 10 out of 13 in NSCLC, 22 out of 24 in renal carcinoma, 8 out of 14 in SCLC cancer and 45 out of 49 in soft tissue tumor, in comparing with corresponding normal tissue.

Stimulation of the T cells using the predicted peptides from INHBB restricted with HLA-A0201 and establishment for CTL lines stimulated with INHBB derived peptides

CTLs for those peptides derived from INHBB were generated according to the protocols set forth in "Materials and Methods" section above. Resulting CTLs having detectable specific CTL activity, as determined by IFN-gamma ELISPOT assay, are shown in Figure 1.

INHBB-A02-9-213 (SEQ ID NO: 1), INHBB-A02-9-174 (SEQ ID NO: 2), INHBB-A02-9-257 (SEQ ID NO: 3), INHBB-A02-9-313 (SEQ ID NO: 4), INHBB-A02-9-139 (SEQ ID NO: 5), INHBB-A02-9-8 (SEQ ID NO: 6), INHBB-A02-9-250 (SEQ ID NO: 7), INHBB-A02-10-179 (SEQ ID NO: 8), INHBB-A02-10-237 (SEQ ID NO: 9), INHBB-A02-10-313 (SEQ ID NO: 10), INHBB-A02-10-173 (SEQ ID NO: 11), INHBB-A02-10-256 (SEQ ID NO: 12), INHBB-A02-10-162 (SEQ ID NO: 13) and INHBB-A02-10-85 (SEQ ID NO: 14) demonstrated potent IFN-gamma production as compared to the control by IFN-gamma ELISPOT assay. Furthermore, the cells in the positive well number #7 stimulated with SEQ ID NO: 2, were expanded and CTL line was established. The CTL line having higher specific CTL activity against the peptide-pulsed target as compared to the activity against target without peptide pulse was determined by IFN-gamma ELISA (Figure 2).

The results herein demonstrate that the CTL line demonstrated potent IFN-gamma production against the target cells pulsed with corresponding peptide as compared to target cells without peptide pulse. In the context of the present invention, the peptides which could establish CTL line were selected as potent CTL stimulation peptide.

[0112] In conclusion, novel HLA-A02 epitope peptides derived from INHBB were identified and demonstrated to be applicable for cancer immunotherapy.

Industrial Applicability

[0113] The present invention describes new TAAs, particularly those derived from INHBB, that induce potent and specific anti-tumor immune responses and have applicability to

a wide array of cancer types. Such TAAs warrant further development as peptide vaccines against diseases associated with INHBB, e.g., cancer, more particularly, cholangio cellular carcinoma, esophageal cancer, non-small cell lung cancer (NSCLC), renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor.

[0114] While the invention is herein described in detail and with reference 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 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 invention, the metes and bounds of which are defined by the appended claims.

Claims

[Claim 1] An isolated peptide derived from SEQ ID NO: 16, wherein said peptide comprises an amino acid sequence selected from the group consisting of:
(a) SEQ ID NOs: 1 to 14; and
(b) SEQ ID NOs: 1 to 14, wherein 1, 2, or several amino acids are substituted, inserted, deleted and/or added,
and has cytotoxic T lymphocyte (CTL) inducibility.

[Claim 2] The peptide as set forth in claim 1, wherein the peptide consists of less than 15 amino acid residues.

[Claim 3] The peptide as set forth in claim 2, wherein the peptide is a nonapeptide or a decapeptide.

[Claim 4] The peptide as set forth in claim 1 to 3, wherein the peptide, comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 14, has one or both of the following characteristics:
(a) the second amino acid from the N-terminus is selected from the group of leucine or methionine, and
(b) the C-terminal amino acid is selected from the group of valine or leucine.

[Claim 5] An isolated polynucleotide encoding one or more peptides as set forth in any one of claims 1 to 4.

[Claim 6] A pharmaceutical agent comprising as active ingredient selected from the group consisting of:
(a) one or more peptides as set forth in any one of claims 1 to 4;
(b) one or more polynucleotides encoding the peptide
(c) one or more antigen-presenting cells and/or exosomes, which present a complex formed between a HLA antigen and a peptide as set forth in any one of claims 1 to 4 on its surface,
(d) one or more CTLs induced against a peptide as set forth in any one of claims 1 to 4; and
(e) combinations thereof,
in combination with a pharmacologically acceptable carrier formulated for a purpose selected from the group consisting of:
(i) treatment of cancer,
(ii) prophylaxis of cancer,
(iii) preventing postoperative recurrence of cancer, and
(iv) combinations thereof.

[Claim 7] The pharmaceutical agent as set forth in claim 6, formulated for administration to a subject whose HLA antigen is HLA-A02.

[Claim 8] The pharmaceutical agent of claim 6, wherein said cancer is selected from the group consisting of cholangio cellular carcinoma, esophageal cancer, non-small cell lung cancer (NSCLC), renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor.

[Claim 9] The pharmaceutical agent of claim 6, wherein said agent is formulated as a vaccine.

[Claim 10] A method for inducing an antigen-presenting cell with high CTL inducibility, wherein the method comprises the step selected form the group consisting of:
(a) contacting an antigen-presenting cell with a peptide set forth in any one of claims 1 to 4; and
(b) introducing a polynucleotide encoding the peptide in an expressible form into an antigen-presenting cell.

[Claim 11] A method for inducing CTL, wherein the method comprises the step selected from the group consisting of:
(a) contacting CD8-positive T cells with antigen-presenting cells and/or exosomes which present a complex formed between an HLA antigen and a peptide as set forth in any one of claims 1 to 4.; and
(b) introducing a polynucleotide encoding a polypeptide which is capable of forming a TCR subunit recognizing a complex formed between an HLA antigen and a peptide as set for the in any one of claims 1 to 4 into a CD8-positive T cell.

[Claim 12] An isolated CTL which targets any of the peptides set forth in any one of claims 1 to 4.

[Claim 13] An isolated CTL which is induced by a peptide as set forth in any one of claims 1 to 4.

[Claim 14] The CTL as set forth in claim 12 or 13, which is capable of recognizing a complex formed between an HLA antigen and a peptide as set forth in any one of claims 1 to 4 on a cell surface.

[Claim 15] The CTL as set forth in claims 12 to 14, which is induced by the method as set forth in claim 11.

[Claim 16] An isolated antigen-presenting cell, which presents on its surface a complex formed between an HLA antigen and a peptide as set forth in any one of claims 1 to 4.

[Claim 17] The antigen-presenting cell of claim 16, which is induced by the method of claim 10.

[Claim 18] The antigen-presenting cell as set forth in claim 16 or 17, wherein the HLA antigen is HLA-A02.

[Claim 19] An agent for inducing an immune response against a cancer in a subject, wherein said agent comprises an active ingredient selected form the group consisting of:
(a) one or more peptides as set forth in any one of claims 1 to 4;
(b) one or more polynucleotides encoding the peptide in an expressible form;
(c) one or more antigen-presenting cells and/or exosomes, which antigen-presenting cells and exosomes present a complex formed between a HLA antigen and a peptide as set forth in any one of claims 1 to 4 on its surface;
(d) one or more CTLs induced against a peptide as set forth in any one of claims 1 to 4; and
(e) combinations thereof.

[Claim 20] A method of inducing an immune response against a cancer in a subject, said method comprising the step of administering to said subject an agent comprising an active ingredient selected from the group consisting of:
(a) one or more peptides as set forth in any one of claims 1 to 4;
(b) one or more polynucleotides encoding the peptide in an expressible form;
(c) one or more antigen-presenting cells and/or exosomes, which antigen-presenting cells and exosomes present a complex formed between a HLA antigen and a peptide as set forth in any one of claims 1 to 4 on its surface;
(d) one or more CTLs induced against a peptide as set forth in any one of claims 1 to 4; and
(e) combinations thereof,
and a pharmaceutically acceptable carrier

[Claim 21] The method as set forth in claim 20, wherein cancer is selected from the group consisting of cholangio cellular carcinoma, esophageal cancer, non-small cell lung cancer (NSCLC), renal carcinoma, small cell lung cancer (SCLC) and soft tissue tumor.

[Claim 22] The method as set forth in claim 20 or 21, wherein the subject has HLA A02.

[Claim 23] An agent for inducing CTL, wherein the agent comprises one or more peptides of any one of claims 1 to 4, a polynucleotide encoding the

peptide, or an isolated antigen-presenting cell of any or of claims 16 to 18.

SEQUENCE LISTING

<110> ONCOTHERAPY SCIENCE, INC.

<120> INHBB EPITOPE PEPTIDES AND VACCINES CONTAINING THE SAME

<130> ONC-A0814P

<150> US 61/089,973
<151> 2008-08-19

<160> 16

<170> PatentIn version 3.5

<210> 1
<211> 9
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 1

Asn Met Val Glu Lys Arg Val Asp Leu
1 5

<210> 2
<211> 9
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 2

Val Gln Ala Ser Leu Trp Leu Tyr Leu
1 5

<210> 3
<211> 9
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 3

Glu Leu Ala Val Val Pro Val Phe Val
1 5

<210> 4
<211> 9
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 4

Arg Leu Ile Gly Trp Asn Asp Trp Ile
1 5

<210> 5
<211> 9
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 5

Arg Val Ser Glu Ile Ile Ser Phe Ala
1 5

<210> 6
<211> 9
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 6

Ala Leu Gly Ala Ala Cys Leu Leu Leu
1 5

<210> 7
<211> 9
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 7

Val Gln Cys Asp Ser Cys Gln Glu Leu
1 5

<210> 8
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 8

Trp Leu Tyr Leu Lys Leu Leu Pro Tyr Val
1 5 10

<210> 9
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 9

Ala Leu Phe Glu Arg Gly Glu Arg Arg Leu
1 5 10

<210> 10

<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 10

Arg Leu Ile Gly Trp Asn Asp Trp Ile Ile
1 5 10

<210> 11
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 11

Val Val Gln Ala Ser Leu Trp Leu Tyr Leu
1 5 10

<210> 12
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 12

Gln Glu Leu Ala Val Val Pro Val Phe Val
1 5 10

<210> 13
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 13

Phe Ile Ser Asn Glu Gly Asn Gln Asn Leu
1 5 10

<210> 14
<211> 10
<212> PRT
<213> Artificial

<220>
<223> An artificially synthesized peptide

<400> 14

Arg Leu Gln Met Arg Gly Arg Pro Asn Ile
1 5 10

<210> 15
<211> 3218

<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (47)..(1270)

<400> 15
```
actcggctcg cctcgcggcg ggcgccctcg tcgccagcgg cgcacc atg gac ggg          55
                                                      Met Asp Gly
                                                      1

ctg ccc ggt cgg gcg ctg ggg gcc gcc tgc ctt ctg ctg ctg gcg gcc        103
Leu Pro Gly Arg Ala Leu Gly Ala Ala Cys Leu Leu Leu Leu Ala Ala
    5                   10                  15

ggc tgg ctg ggg cct gag gcc tgg ggc tca ccc acg ccc ccg ccg acg        151
Gly Trp Leu Gly Pro Glu Ala Trp Gly Ser Pro Thr Pro Pro Pro Thr
20                  25                  30                  35

cct gcc gcg ccg ccg cca ccc ccg cca ccc gga tcc ccg ggt ggc tcg        199
Pro Ala Ala Pro Pro Pro Pro Pro Pro Gly Ser Pro Gly Gly Ser
                40                  45                  50

cag gac acc tgt acg tcg tgc ggc ggc ttc cgg cgg cca gag gag ctc        247
Gln Asp Thr Cys Thr Ser Cys Gly Gly Phe Arg Arg Pro Glu Glu Leu
            55                  60                  65

ggc cga gtg gac ggc gac ttc ctg gag gcg gtg aag cgg cac atc ttg        295
Gly Arg Val Asp Gly Asp Phe Leu Glu Ala Val Lys Arg His Ile Leu
        70                  75                  80

agc cgc ctg cag atg cgg ggc cgg ccc aac atc acg cac gcc gtg cct        343
Ser Arg Leu Gln Met Arg Gly Arg Pro Asn Ile Thr His Ala Val Pro
    85                  90                  95

aag gcc gcc atg gtc acg gcc ctg cgc aag ctg cac gcg ggc aag gtg        391
Lys Ala Ala Met Val Thr Ala Leu Arg Lys Leu His Ala Gly Lys Val
100                 105                 110                 115

cgc gag gac ggc cgc gtg gag atc ccg cac ctc gac ggc cac gcc agc        439
Arg Glu Asp Gly Arg Val Glu Ile Pro His Leu Asp Gly His Ala Ser
                120                 125                 130

ccg ggc gcc gac ggc cag gag cgc gtt tcc gaa atc atc agc ttc gcc        487
Pro Gly Ala Asp Gly Gln Glu Arg Val Ser Glu Ile Ile Ser Phe Ala
            135                 140                 145

gag aca gat ggc ctc gcc tcc tcc cgg gtc cgc cta tac ttc ttc atc        535
Glu Thr Asp Gly Leu Ala Ser Ser Arg Val Arg Leu Tyr Phe Phe Ile
        150                 155                 160

tcc aac gaa ggc aac cag aac ctg ttt gtg gtc cag gcc agc ctg tgg        583
Ser Asn Glu Gly Asn Gln Asn Leu Phe Val Val Gln Ala Ser Leu Trp
    165                 170                 175

ctt tac ctg aaa ctc ctg ccc tac gtc ctg gag aag ggc agc cgg cgg        631
Leu Tyr Leu Lys Leu Leu Pro Tyr Val Leu Glu Lys Gly Ser Arg Arg
180                 185                 190                 195

aag gtg cgg gtc aaa gtg tac ttc cag gag cag ggc cac ggt gac agg        679
Lys Val Arg Val Lys Val Tyr Phe Gln Glu Gln Gly His Gly Asp Arg
                200                 205                 210

tgg aac atg gtg gag aag agg gtg gac ctc aag cgc agc ggc tgg cat        727
Trp Asn Met Val Glu Lys Arg Val Asp Leu Lys Arg Ser Gly Trp His
            215                 220                 225

acc ttc cca ctc acg gag gcc atc cag gcc ttg ttt gag cgg ggc gag        775
Thr Phe Pro Leu Thr Glu Ala Ile Gln Ala Leu Phe Glu Arg Gly Glu
```

```
        230                 235                 240

cgg cga ctc aac cta gac gtg cag tgt gac agc tgc cag gag ctg gcc        823
Arg Arg Leu Asn Leu Asp Val Gln Cys Asp Ser Cys Gln Glu Leu Ala
    245                 250                 255

gtg gtg ccg gtg ttc gtg gac cca ggc gaa gag tcg cac cgg ccc ttt        871
Val Val Pro Val Phe Val Asp Pro Gly Glu Glu Ser His Arg Pro Phe
260                 265                 270                 275

gtg gtg gtg cag gct cgg ctg ggc gac agc agg cac cgc att cgc aag        919
Val Val Val Gln Ala Arg Leu Gly Asp Ser Arg His Arg Ile Arg Lys
                280                 285                 290

cga ggc ctg gag tgc gat ggc cgg acc aac ctc tgt tgc agg caa cag        967
Arg Gly Leu Glu Cys Asp Gly Arg Thr Asn Leu Cys Cys Arg Gln Gln
            295                 300                 305

ttc ttc att gac ttc cgc ctc atc ggc tgg aac gac tgg atc ata gca       1015
Phe Phe Ile Asp Phe Arg Leu Ile Gly Trp Asn Asp Trp Ile Ile Ala
        310                 315                 320

ccc acc ggc tac tac ggg aac tac tgt gag ggc agc tgc cca gcc tac       1063
Pro Thr Gly Tyr Tyr Gly Asn Tyr Cys Glu Gly Ser Cys Pro Ala Tyr
    325                 330                 335

ctg gca ggg gtc ccc ggc tct gcc tcc tcc ttc cac acg gct gtg gtg       1111
Leu Ala Gly Val Pro Gly Ser Ala Ser Ser Phe His Thr Ala Val Val
340                 345                 350                 355

aac cag tac cgc atg cgg ggt ctg aac ccc ggc acg gtg aac tcc tgc       1159
Asn Gln Tyr Arg Met Arg Gly Leu Asn Pro Gly Thr Val Asn Ser Cys
                360                 365                 370

tgc att ccc acc aag ctg agc acc atg tcc atg ctg tac ttc gat gat       1207
Cys Ile Pro Thr Lys Leu Ser Thr Met Ser Met Leu Tyr Phe Asp Asp
            375                 380                 385

gag tac aac atc gtc aag cgg gac gtg ccc aac atg att gtg gag gag       1255
Glu Tyr Asn Ile Val Lys Arg Asp Val Pro Asn Met Ile Val Glu Glu
        390                 395                 400

tgc ggc tgc gcc tga cagtgcaagg caggggcacg gtggtggggc acggagggca       1310
Cys Gly Cys Ala
    405

gtcccgggtg ggcttcttcc agcccccgcg ggaacggggg tacacggtgg gctgagtaca      1370

gtcattctgt tgggctgtgg agatagtgcc agggtgcggc ctgagatatt tttctacagc      1430

ttcatagagc aaccagtcaa aaccagagcg agaaccctca actgacatga aatactttaa      1490

aatgcacacg tagccacgca cagccagacg catcctgcca cccacacagc agcctccagg      1550

ataccagcaa atggatgcgg tgacaaatgg cagcttagct acaaatgcct gtcagtcgga      1610

gagaatgggg tgagcagcca ccattcccac cagctggccc ggccactctg aattgcgcct      1670

tccgagcaca cataaaagca caaagacaga gacgcagaga gagagagaga gccacggaga      1730

ggaaaagcag atgcaggggt ggggagcgca gctcggcgga ggctgcgtgt gccccgtggc      1790

ttttaccagg cctgctctgc ctggctcgat gtctgcttct tccccagcct gggatccttc      1850

gtgcttcaag gcctggggag cctgtccttc catgcccttg tcgagggaaa gagacccaga      1910

aaggacacaa cccgtcagag acctgggagc aggggcaatg accgtttgac tgtttgtggc      1970

ttgggcctct gacatgactt atgtgtgtgt gtgtttttgg ggtggggagg gagggagaga      2030

agaggggget aaatttgatg ctttaactga tctccaacag ttgacaggtc atccttgcca      2090
```

gttgtataac tgaaaaagga cttttctacc aggtatgacc ttttaagtga aaatctgaat 2150

tgttctaaat ggaaagaaaa aaagttgcaa tctgtgccct tcattgggga cattcctcta 2210

ggactggttt ggggacgggt gggaatgacc cctaggcaag gggatgagac cgcaggagga 2270

aatggcgggg aggaggcatt cttgaactgc tgaggatggg gggtgtcccc tcagcggagg 2330

ccaagggagg ggagcagcct agttggtctt ggagagatgg ggaaggcttt cagctgattt 2390

gcagaagttg cccatgtggg ccccagccat cagggctggc cgtggacgtg gcccctgccc 2450

actcacctgc ccgcctgccc gcccgcccgc atagcacttg cagacctgcc tgaacgcaca 2510

tgacatagca cttgccgatc tgcgtgtgtc cagaagtggc ccttggccga gcgccgaact 2570

cgctcgccct ctagatgtcc aagtgccacg tgaactatgc aatttaaagg gttgacccac 2630

actagacgaa actggactcg tacgactctt tttatatttt ttatacttga aatgaaatcc 2690

tttgcttctt ttttaagcga atgattgctt ttaatgtttg cactgattta gttgcatgat 2750

tagtcagaaa ctgccatttg aaaaaaagtt atttttatag cagcaaaaaa aaaaaaaaaa 2810

gaatacagtt aaatgtatta tacataattt tggaaccaaa gaggccaaca gatcagtttt 2870

aattttatta gacggtgagg ccatctgaga tgaggtggac gttctgagca gtcccttgag 2930

tggcctgcca acgtttcagg gtatgaatgg attttgttta ttcggtttga tgtgtctttt 2990

ccatccttac acacccagaa ggtagagtaa aaatgactat gatagaatgc aggtgtgtat 3050

ccttaaatcc tcatctttat gtttatttaa taaagctccc cttagattct gtttcataat 3110

aatttaaaac caaacaattt tcccatagac ttgctgttaa agtattgtac gtttgtgtac 3170

agtttaagaa aataaaagat tgagtgccac gggaaaaaaa aaaaaaaa 3218

<210> 16
<211> 407
<212> PRT
<213> Homo sapiens

<400> 16

Met Asp Gly Leu Pro Gly Arg Ala Leu Gly Ala Ala Cys Leu Leu Leu
1 5 10 15

Leu Ala Ala Gly Trp Leu Gly Pro Glu Ala Trp Gly Ser Pro Thr Pro
20 25 30

Pro Pro Thr Pro Ala Ala Pro Pro Pro Pro Pro Pro Pro Gly Ser Pro
35 40 45

Gly Gly Ser Gln Asp Thr Cys Thr Ser Cys Gly Gly Phe Arg Arg Pro
50 55 60

Glu Glu Leu Gly Arg Val Asp Gly Asp Phe Leu Glu Ala Val Lys Arg
65 70 75 80

His Ile Leu Ser Arg Leu Gln Met Arg Gly Arg Pro Asn Ile Thr His
85 90 95

```
Ala Val Pro Lys Ala Ala Met Val Thr Ala Leu Arg Lys Leu His Ala
            100                 105                 110

Gly Lys Val Arg Glu Asp Gly Arg Val Glu Ile Pro His Leu Asp Gly
        115                 120                 125

His Ala Ser Pro Gly Ala Asp Gly Gln Glu Arg Val Ser Glu Ile Ile
    130                 135                 140

Ser Phe Ala Glu Thr Asp Gly Leu Ala Ser Ser Arg Val Arg Leu Tyr
145                 150                 155                 160

Phe Phe Ile Ser Asn Glu Gly Asn Gln Asn Leu Phe Val Val Gln Ala
                165                 170                 175

Ser Leu Trp Leu Tyr Leu Lys Leu Leu Pro Tyr Val Leu Glu Lys Gly
            180                 185                 190

Ser Arg Arg Lys Val Arg Val Lys Val Tyr Phe Gln Glu Gln Gly His
        195                 200                 205

Gly Asp Arg Trp Asn Met Val Glu Lys Arg Val Asp Leu Lys Arg Ser
    210                 215                 220

Gly Trp His Thr Phe Pro Leu Thr Glu Ala Ile Gln Ala Leu Phe Glu
225                 230                 235                 240

Arg Gly Glu Arg Arg Leu Asn Leu Asp Val Gln Cys Asp Ser Cys Gln
                245                 250                 255

Glu Leu Ala Val Val Pro Val Phe Val Asp Pro Gly Glu Glu Ser His
            260                 265                 270

Arg Pro Phe Val Val Val Gln Ala Arg Leu Gly Asp Ser Arg His Arg
        275                 280                 285

Ile Arg Lys Arg Gly Leu Glu Cys Asp Gly Arg Thr Asn Leu Cys Cys
    290                 295                 300

Arg Gln Gln Phe Phe Ile Asp Phe Arg Leu Ile Gly Trp Asn Asp Trp
305                 310                 315                 320

Ile Ile Ala Pro Thr Gly Tyr Tyr Gly Asn Tyr Cys Glu Gly Ser Cys
                325                 330                 335

Pro Ala Tyr Leu Ala Gly Val Pro Gly Ser Ala Ser Ser Phe His Thr
            340                 345                 350

Ala Val Val Asn Gln Tyr Arg Met Arg Gly Leu Asn Pro Gly Thr Val
        355                 360                 365

Asn Ser Cys Cys Ile Pro Thr Lys Leu Ser Thr Met Ser Met Leu Tyr
    370                 375                 380
```

```
Phe Asp Asp Glu Tyr Asn Ile Val Lys Arg Asp Val Pro Asn Met Ile
385                 390                 395                 400

Val Glu Glu Cys Gly Cys Ala
                405
```