Description

Title of Invention: SEMA5B PEPTIDES AND VACCINES CONTAINING THE SAME

Technical Field

[0001] The present invention relates to the field of biological science, more specifically to the field of cancer therapy. In particular, the present invention relates to novel peptides that are effective as cancer vaccines, as well as drugs for either or both of the treatment and/or prophylaxis of tumors.

[0002] Priority

The present application claims the benefit of U.S. Provisional Applications No. 61/733,279, filed on December 4, 2012, the entire contents of which are incorporated by reference herein.

Background Art

[0003] CD8 positive cytotoxic T lymphocytes (CTLs) have been shown to recognize epitope peptides derived from the tumor-associated antigens (TAAs) found on the 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 1-2). Some of these TAAs are currently undergoing clinical development as immunotherapeutic targets.

[0004] Favorable TAAs are indispensable for the proliferation and survival of cancer cells. The use of such TAAs as targets for immunotherapy may minimize the well-described risk of immune escape of cancer cells attributable to deletion, mutation, or down-regulation of TAAs as a consequence of therapeutically driven immune selection. Accordingly, the identification of new TAAs capable of inducing potent and specific anti-tumor immune responses warrants further development. Thus, the clinical application of peptide vaccination strategies for various types of cancer in ongoing (NPL 3-10). To date, there have been several reports of clinical trials using these TAA derived peptides. Unfortunately, these cancer vaccine trials have to date yielded only a low objective response rate (NPL 11-13). Accordingly, there remains a need for new TAAs suitable for use as immunotherapeutic targets.

[0005] SEMA5B is a member of the semaphorin protein family, a class of secreted and membranal proteins that are involved in axonal guidance during neural development (NPL 14). Recent study suggested that the functions of semaphorin family protein are related not only to nervous system but to organogenesis, angiogenesis and a development of cancer.

[0006] In the process of clarifying the molecular mechanism of renal cell carcinoma (RCC) by gene-expression profile analysis using cDNA microarray consisting of 23,040 genes, SEMA5B was discovered to be frequently up-regulated in RCC.

[0007] RT-PCR analysis of this gene in a cDNA sample derived from RCC patient demonstrated that SEMA5B was up-regulated in all of the RCC samples.

Subsequent Northern blot analysis using an SEMA5B cDNA fragment as a probe revealed an SEMA5B transcript that was highly expressed in RCC tissues but was hardly detectable in normal human tissues except fetal brain and kidney (PTL 1).

Furthermore, knockdown of SEMA5B by siRNA in RCC cell lines attenuated the growth of RCC cells (NPL 15).

Citation List

Patent Literature

[0008] [PTL 1] WO2007/013575

Non Patent Literature

[0009] [NPL 1] Boon T, Int J Cancer 1993, 54(2): 177-80

[NPL 2] Boon T & van der Bruggen P, J Exp Med 1996, 183(3): 725-9

[NPL 3] Harris CC, J Natl Cancer Inst 1996, 88(20): 1442-55

[NPL 4] Butterfield LH et al., Cancer Res 1999, 59(13): 3134-42

[NPL 5] Vissers JL et al., Cancer Res 1999, 59(21): 5554-9

[NPL 6] van der Burg SH et al., J Immunol 1996, 156(9): 3308-14

[NPL 7] Tanaka F et al., Cancer Res 1997, 57(20): 4465-8

[NPL 8] Fujie T et al., Int J Cancer 1999, 80(2): 169-72

[NPL 9] Kikuchi M et al., Int J Cancer 1999, 81(3): 459-66

[NPL 10] Oiso M et al., Int J Cancer 1999, 81(3): 387-94

[NPL 11] Belli F et al., J Clin Oncol 2002, 20(20): 4169-80

[NPL 12] Coulie PG et al., Immunol Rev 2002, 188: 33-42

[NPL 13] Rosenberg SA et al., Nat Med 2004, 10(9): 909-15

[NPL 14] O'Connor TP et.al., Neural Dev. 2009,23(4)18

[NPL 15] Hirota E. et.al., Int J Oncol. 2006, 29(4):799-827

Summary of Invention

[0010] The present invention is based, at least in part, on the discovery of novel peptides that may serve as suitable targets of immunotherapy. Because TAAs are generally perceived by the immune system as "self" and therefore often have no innate immunogenicity, the discovery of appropriate targets is still of importance. In the course of the present invention, SEMA5B (a typical amino acid sequence shown in SEQ ID NO: 75, 78 or 80; a typical nucleotide sequence shown in SEQ ID NO: 74, 76, 77 or 79 (GenBank Accession No. NM_001031702, NM_001256346, NM_001256347 or

NM_001256348)) is demonstrated to be specifically over-expressed in cancers, examples of which include, but are not limited to, esophageal cancer, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC) and small cell lung cancer (SCLC) (Table 1). Thus, the present invention focuses on SEMA5B as a candidate target for cancer/tumor immunotherapy.

[0011] To that end, the present invention is directed, at least in part, to the identification of specific epitope peptides that possess the ability to induce cytotoxic T lymphocytes (CTLs) specific to SEMA5B among peptides derived SEMA5B. As discussed in greater detail below, peripheral blood mononuclear cells (PBMCs) obtained from a healthy donor were stimulated using HLA-A*2402 binding candidate peptides derived from SEMA5B. CTL lines were then established with specific cytotoxicity against the HLA-A24 positive target cells pulsed with each of candidate peptides. The results herein demonstrate that these peptides are HLA-A24 restricted epitope peptides that can induce potent and specific immune responses against cells expressing SEMA5B. These results further indicate that SEMA5B is strongly immunogenic and that the epitopes thereof are effective targets for cancer/tumor immunotherapy.

[0012] Accordingly, it is an object of the present invention to provide isolated peptides that can bind to an HLA antigen and include an amino acid sequence derived from the SEMA5B (SEQ ID NO: 75, 78 or 80). Such peptides are expected to have CTL inducibility and, thus, can be used to induce CTLs in vitro or ex vivo, or to be directly administered to a subject so as to induce in vivo immune responses against cancers, examples of which include, but are not limited to, esophageal cancer, NSCLC, RCC and SCLC.

[0013] The peptides of the present invention are generally less than 15, 14, 13, 12, 11, or 10 amino acids in length. Preferred peptides are nonapeptides and decapeptides, more preferably, nonapeptides and decapeptides having an amino acid sequence selected from among SEQ ID NOs: 2 to 69. Of these, the peptides having an amino sequence selected from among SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54 are particularly preferred.

[0014] The present invention also contemplates modified peptides having an amino acid sequence in which one, two or more amino acids are substituted, deleted, inserted and/or added in an amino acid sequence selected from among SEQ ID NOs: 2 to 69, provided the resulting modified peptides retain the requisite CTL inducibility and HLA binding ability of the original unmodified peptide.

[0015] In one embodiment, when the original peptide is a 9-mer (e.g., one of SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, and 20), the size of the modified peptide is preferably in the range of 9 to 40 amino acids, such as in the range of 9 to 20 amino acids, for example in the range of 9 to 15 amino acids. Likewise, when the original peptide is a 10-mer (e.g., one

of SEQ ID NOs: 40, 41, 47 and 54), the size of the modified peptide is preferably in the range of 10 to 40 amino acids, such as in the range of 10 to 20 amino acids, for example in the range of 10 to 15 amino acids.

[0016] The present invention further encompasses isolated polynucleotides encoding any one of the peptides of the present invention. These polynucleotides can be used to induce or prepare antigen presenting cells (APCs) having CTL inducibility. Like the peptides of the present invention, such APCs can be administered to a subject for inducing immune responses against cancers.

[0017] When administered to a subject, the peptides of the present invention can be presented on the surface of APCs so as to induce CTLs targeting the respective peptides. Therefore, one object of the present invention is to provide agents or compositions including one or more peptides of the present invention, or one or more polynucleotides encoding such peptides. The agent or composition may be used for inducing a CTL. Such agents or compositions can be used for the treatment and/or prophylaxis of a cancer, and/or the prevention of a metastasis or post-operative recurrence thereof. Examples of targeted cancers contemplated by the present invention include, but are not limited to, esophageal cancer, NSCLC, RCC and SCLC.

[0018] The present invention further contemplates pharmaceutical compositions or agents that include one or more peptides or polynucleotides of the present invention. The pharmaceutical composition or agent is preferably formulated for use in the treatment and/or prophylaxis of a cancer, more particularly a primary cancer, and/or prevention of metastasis or postoperative recurrence thereof. Instead of or in addition to the peptides or polynucleotides of the present invention, the pharmaceutical agents or compositions of the present invention may include as active ingredients APCs or exosomes that present any of the peptides of the present invention.

[0019] The peptides or polynucleotides of the present invention may be used to induce APCs that present on the surface a complex of an HLA antigen and a peptide of the present invention, for example, by contacting APCs derived from a subject with a peptide of the present invention or introducing a polynucleotide encoding a peptide of the present invention into APCs. Such APCs have an ability to inducing CTLs that specifically recognize cells that present target peptides on the surface and are useful for cancer immunotherapy. Accordingly, the present invention encompasses the methods for inducing APCs with CTL inducibility as well as the APCs obtained by such methods.

In addition, the present invention also encompasses the agents or compositions for inducing APCs having CTL inducibility, such agents or compositions including any peptides or polynucleotides of the present invention.

[0020] It is a further object of the present invention to provide methods for inducing CTLs,

such methods including the step of co-culturing CD8 positive T cells with APCs presenting on its surface a complex of an HLA antigen and a peptide of the present invention, the step of co-culturing CD8 positive T cells with exosomes presenting on its surface a complex of an HLA antigen and a peptide of the present invention, or the step of introducing a polynucleotide encoding both of T cell receptor (TCR) subunits or polynucleotides encoding each of TCR subunits, wherein the TCR can bind to a complex of the present invention and an HLA antigen presented on cell surface. CTLs obtained by such methods can find use in the treatment and/or prevention of cancers, examples of which include, but are not limited to, esophageal cancer, NSCLC, RCC and SCLC. Accordingly, the present invention encompasses both the methods for inducing CTLs and the CTLs obtained by such methods

Yet another object of the present invention is to provide isolated APCs that present on the surface a complex of an HLA antigen and a peptide of the present invention. The present invention further provides isolated CTLs that target peptides of the present invention. Such CTLs may be also defined as CTLs that can recognize (or bind to) a complex of a peptide of the present invention and an HLA antigen on the cell surface. These APCs and CTLs may be used for cancer immunotherapy.

[0021] It is yet another object of the present invention to provide methods for inducing an immune response against a cancer in a subject in need thereof, such methods including the step of administering to the subject an agent or composition that includes at least one component selected from among (a) a peptide of the present invention or a polynucleotide encoding such a peptide, (b) an APC or exosome presenting such peptides and (c) a CTL that can recognize such a cell presenting a peptide of the present invention on its surface.

[0022] One aspect of the present invention pertains to a peptide of the present invention, an agent or composition comprising such a peptide for use as a medicament.

The applicability of the present invention extends to any of a number of diseases relating to or arising from SEMA5B overexpression, such as cancer, examples of which include, but are not limited to, esophageal cancer, NSCLC, RCC and SCLC.

[0023] More specifically, the present invention provides followings:

[1] An isolated peptide having cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide comprises an amino acid sequence (a) or (b) below:

(a) an amino acid sequence of an immunologically active fragment of SEMA5B;

(b) an amino acid sequence in which 1, 2, or several amino acid(s) are substituted, deleted, inserted and/or added in an amino acid sequence of an immunologically active fragment of SEMA5B,

wherein the CTL induced by the peptide has specific cytotoxic activity against a cell that presents a fragment derived from SEMA5B;

[2] The peptide of [1], wherein the peptide comprises an amino acid sequence (a) or (b) below:

(a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54;

(b) an amino acid sequence in which 1, 2, or several amino acid(s) are substituted, deleted, inserted and/or added in the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54;

[3] The peptide of [2], wherein the peptide has one or both of the following characteristics:

(a) the second amino acid from the N-terminus is phenylalanine, tyrosine, methionine or tryptophan; and

(b) the C-terminal amino acid is phenylalanine, leucine, isoleucine, tryptophan or methionine;

[4] The peptide of any one of [1] to [3], wherein the peptide is a nonapeptide or a decapeptide;

[5] An isolated polynucleotide encoding the peptide of any one of [1] to [4];

[6] A composition for inducing a CTL, wherein the composition comprises at least one active ingredient selected from the group consisting of:

(a) the peptide of any one of [1] to [4];

(b) the polynucleotide of [5];

(c) an antigen-presenting cell (APC) that presents the peptide of any one of [1] to [4] on its surface; and

(d) an exosome that presents the peptide of any one of [1] to [4] on its surface;

[7] A pharmaceutical composition for the treatment and/or prophylaxis of cancer, and/or the prevention of a postoperative recurrence thereof, or the induction of an immune response against said cancer, wherein the composition comprises at least one active ingredient selected from the group consisting of:

(a) the peptide of any one of [1] to [4];

(b) the polynucleotide of [5];

(c) an APC that presents the peptide of any one of [1] to [4] on its surface;

(d) an exosome that presents the peptide of any one of [1] to [4] on its surface; and

(e) a CTL that can recognize a cell presenting the peptide of any one of [1] to [4];

[8] The pharmaceutical composition of [7], wherein the pharmaceutical composition is formulated for the administration to a subject whose HLA antigen is HLA-A24;

[9] A method for inducing an APC with CTL inducibility, wherein the method comprises the step selected from the group consisting of:

(a) contacting an APC with the peptide of any one of [1] to [4], and

(b) introducing a polynucleotide encoding the peptide of any one of [1] to [4] into an

APC;

[10] A method for inducing a CTL, wherein the method comprises a step selected from the group consisting of:

(a) co-culturing a CD8 positive T cell with an APC that presents on its surface a complex of an HLA antigen and the peptide of any one of [1] to [4];

(b) co-culturing a CD8 positive T cell with an exosome that presents on its surface a complex of an HLA antigen and the peptide of any one of [1] to [4]; and

(c) introducing into a CD8 positive T cell a polynucleotide encoding both of T cell receptor (TCR) subunits or polynucleotides encoding each of TCR subunits, wherein the TCR formed by said subunits can bind to a complex of the peptide of any one of [1] to [4] and an HLA antigen on a cell surface;

[11] An isolated APC that presents on its surface a complex of an HLA antigen and the peptide of any one of [1] to [4];

[12] The APC of [11], which is induced by the method of [9];

[13] An isolated CTL that targets the peptide of any one of [1] to [4];

[14] The CTL of [13], which is induced by the method of [10];

[15] A method of inducing an immune response against cancer in a subject, wherein the method comprises the step of administering to the subject a composition comprising the peptide of any one of [1] to [4], or a polynucleotide encoding the peptide;

[16] An antibody or immunologically active fragment thereof against the peptide of any one of [1] to [4];

[17] A vector comprising a nucleotide sequence encoding the peptide of any one of [1] to [4];

[18] A host cell transformed or transfected with a vector of [17];

[19] A diagnostic kit comprising the peptide of any one of [1] to [4], the polynucleotide of [5] or the antibody or immunologically active fragment of [16];

[20] A method of screening for a peptide having an ability to induce a CTL that has specific cytotoxic activity against a cell that presents a fragment derived from SEMA5B, wherein the method comprises the steps of:

(i) providing a candidate sequence consisting of an amino acid sequence modified by substituting, deleting, inserting and/or adding one, two or several amino acid residues to an original amino acid sequence, wherein the original amino acid sequence is selected from the group consisting of SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54;

(ii) selecting a candidate sequence that does not have substantial significant homology with the peptides derived from any known human gene products other than SEMA5B;

(iii) contacting a peptide consisting of the candidate sequence selected in step (ii) with

an antigen presenting cell;

(iv) contacting the antigen presenting cell of step (iii) with a CD8 positive T cell; and

(v) identifying the peptide of which CTL inducibility is same to or higher than a peptide consisting of the original amino acid sequence;

[21] A pharmaceutical composition comprising a peptide of any one of [1] to [4];

[22] A peptide of any one of [1] to [4] for use as a medicament; and

[23] A polynucleotide of [5] or a vector of [17] for use as a medicament.

[0024] Alternatively, in another embodiment, the present invention also provides following peptides and use thereof:

[1] An isolated peptide of less than 15 amino acids in length having cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide comprises an amino acid sequence (a) or (b) below:

(a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 41, 2, 3, 4, 8, 9, 10, 13, 20, 40, 47 and 54;

(b) an amino acid sequence in which 1, 2, or several amino acid(s) are substituted, deleted, inserted and/or added in the amino acid sequence selected from the group consisting of SEQ ID NOs: 41, 2, 3, 4, 8, 9, 10, 13, 20, 40, 47 and 54.

[2] The peptide of [1], wherein the peptide has one or both of the following characteristics:

(a) the second amino acid from the N-terminus is phenylalanine, tyrosine, methionine or tryptophan; and

(b) the C-terminal amino acid is phenylalanine, leucine, isoleucine, tryptophan or methionine;

[3] The peptide of [1] or [2], wherein the peptide is a nonapeptide or a decapeptide.

[0025] Objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying figures and examples. It is to be understood that both the foregoing summary of the present invention and the following detailed description are of exemplified embodiments, and not restrictive of the present invention or other alternate embodiments of the present invention.

[0026] In particular, while the invention is described herein with reference to a number of specific embodiments, it will be appreciated that the description is illustrative of the invention and is not constructed as limiting of the invention. Various modifications and applications may occur to those who are skilled in the art, without departing from the spirit and the scope of the invention, as described by the appended claims. Likewise, other objects, features, benefits and advantages of the present invention will be apparent from this summary and certain embodiments described below, and will be readily apparent to those skilled in the art. Such objects, features, benefits and ad-

vantages will be apparent from the above in conjunction with the accompanying examples, data, figures and all reasonable inferences to be drawn therefrom, alone or with consideration of the references incorporated herein.

Brief Description of Drawings

[0027] 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 that follows.

[0028] [fig.1-1]Figure 1 is composed of a series of photographs, (a) to (m), showing the results of interferon (IFN)-gamma enzyme-linked immunospot (ELISPOT) assay on CTLs that were induced with peptides derived from SEMA5B. The CTLs in the well number #7 induced with SEMA5B-A24-9-512 (SEQ ID NO: 2) (a), in #3 with SEMA5B-A24-9-1010 (SEQ ID NO: 3) (b), in #3 with SEMA5B-A24-9-196 (SEQ ID NO: 4) (c), in #4 with SEMA5B-A24-9-723 (SEQ ID NO: 8) (d), in #5 with SEMA5B-A24-9-280 (SEQ ID NO: 9) (e), and in #3 with SEMA5B-A24-9-293 (SEQ ID NO: 10) (f) showed potent IFN-gamma production as compared with the control, respectively. The square on the well of these pictures indicates that the cells from corresponding well were expanded to establish CTL lines. In contrast, as is typical of negative data, no specific IFN-gamma production was observed from the CTL stimulated with SEMA5B-A24-9-247 (SEQ ID NO: 1) (m). In the figures, "+" indicates the IFN-gamma production against target cells pulsed with the appropriate peptide, and "-" indicates the IFN-gamma production against target cells not pulsed with any peptides.

[0029] [fig.1-2]Figure 1 is composed of a series of photographs, (a) to (m), showing the results of interferon (IFN)-gamma enzyme-linked immunospot (ELISPOT) assay on CTLs that were induced with peptides derived from SEMA5B. The CTLs in the well number #6 induced with SEMA5B-A24-9-470 (SEQ ID NO: 13) (g), in #3 with SEMA5B-A24-9-558 (SEQ ID NO: 20) (h), in #4 with SEMA5B-A24-10-354 (SEQ ID NO: 40) (i), in #6 with SEMA5B-A24-10-290 (SEQ ID NO: 41) (j), in #5 with SEMA5B-A24-10-1044 (SEQ ID NO: 47) (k) and in #4 with SEMA5B-A24-10-489 (SEQ ID NO: 54) (l) showed potent IFN-gamma production as compared with the control, respectively. The square on the well of these pictures indicates that the cells from corresponding well were expanded to establish CTL lines. In contrast, as is typical of negative data, no specific IFN-gamma production was observed from the CTL stimulated with SEMA5B-A24-9-247 (SEQ ID NO: 1) (m). In the figures, "+" indicates the IFN-gamma production against target cells pulsed with the appropriate peptide, and "-" indicates the IFN-gamma production against target cells not pulsed

with any peptides.

[0030] [fig.2]Figure2 is composed of a series of line graphs, (a) to (d), showing the IFN-gamma production of the CTL lines stimulated with SEMA5B-A24-9-512 (SEQ ID NO: 2) (a), SEMA5B-A24-9-1010 (SEQ ID NO: 3) (b), SEMA5B-A24-9-293 (SEQ ID NO: 10) (c) and SEMA5B-A24-10-290 (SEQ ID NO: 41) (d). The quantity of IFN-gamma which CTL produced was measured by IFN-gamma enzyme-linked immunosorbent assay (ELISA). The results demonstrate that CTL lines established by stimulation with each peptide showed potent IFN-gamma production as compared with the control. In the figures, "+" indicates the IFN-gamma production against target cells pulsed with the appropriate peptide, and "-" indicates the IFN-gamma production against target cells not pulsed with any peptides.

[0031] [fig.3]Figure 3 is composed of a series of line graphs, (a) to (c), showing the IFN-gamma production of the CTL clones established by limiting dilution from the CTL lines stimulated with SEMA5B-A24-9-512 (SEQ ID NO: 2) (a), SEMA5B-A24-9-1010 (SEQ ID NO: 3) (b) and SEMA5B-A24-10-290 (SEQ ID NO: 41) (c). The results demonstrate that the CTL clones established by stimulation with each peptide showed potent IFN-gamma production compared with the control. In the figure, "+" indicates the IFN-gamma production against target cells pulsed with the appropriate peptide and "-" indicates the IFN-gamma production against target cells not pulsed with any peptides.

[0032] [fig.4]Figure 4 is a line graph, showing the specific CTL activity of CTL clones against the target cells that express SEMA5B and HLA-A*2402. COS7 cells transfected with HLA-A*2402 or the full length SEMA5B gene were prepared as the controls. The CTL line established with SEMA5B-A24-10-290 (SEQ ID NO: 41) showed specific CTL activity against COS7 cells transfected with both SEMA5B and HLA-A*2402 (-lozenge-). On the other hand, no significant specific CTL activity was detected against target cells expressing either HLA-A*2402 (-triangle-) or SEMA5B (-circle-).

Description of Embodiments

[0033] 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 should be understood that these descriptions are merely illustrative and not intended to be limited. It should also 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. Furthermore, 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.

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 or prior invention.

[0034] 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 the present invention belongs. 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.

[0035] I. Definitions

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

The terms "isolated" and "purified" used in relation with a substance (e.g., peptide, antibody, polynucleotide, etc.) indicates that the substance is substantially free from at least one substance that may else be included in the natural source. Thus, an isolated or purified peptide refers to a peptide that are substantially free of cellular material such as carbohydrate, lipid, or other contaminating proteins from the cell or tissue source from which the peptide is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized.

[0036] The term "substantially free of cellular material" includes preparations of a peptide in which the peptide is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a peptide that is substantially free of cellular material includes preparations of polypeptide having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein"). When the peptide is recombinantly produced, it is also preferably substantially free of culture medium, which includes preparations of peptide with culture medium less than about 20%, 10%, or 5% of the volume of the peptide preparation. When the peptide is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, which includes preparations of peptide with chemical precursors or other chemicals involved in the synthesis of the peptide less than about 30%, 20%, 10%, 5% (by dry weight) of the volume of the peptide preparation. That a particular peptide preparation contains an isolated or purified peptide can be shown, for example, by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining or the like of the gel. In a preferred embodiment, peptides and polynucleotides of the present invention are

isolated or purified.

[0037] 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(s) may be modified residue(s), or non-naturally occurring residue(s), such as artificial chemical mimetic(s) of corresponding naturally occurring amino acid(s), as well as to naturally occurring amino acid polymers.

[0038] The term "oligopeptide" as used herein refers to a peptide which is composed of 20 amino acid residues or fewer, typically 15 amino acid residues or fewer. As used herein, the term "nonapeptide" refers to a peptide which is composed of 9 amino acid residues and the term "decapeptide" refers to a peptide which is composed of 10 amino acid resides.

[0039] 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. Amino acids may be either L-amino acids or D-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 backbone (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.

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.

[0040] The terms "polynucleotide", "oligonucleotide", and "nucleic acid" are used interchangeably herein and, unless otherwise specifically indicated, are referred to by their commonly accepted single-letter codes.

The term "agent" and "composition" are used interchangeably herein to refer to a product that includes specified ingredients in specified amounts, as well as any product that results, directly or indirectly, from combination of the specified ingredients in the specified amounts. Such terms, when used in relation to the modifier "pharmaceutical" (as in "pharmaceutical agent" and "pharmaceutical composition") are intended to encompass a product that includes the active ingredient(s), and the inert ingredient(s) that make up the carrier, as well as any product that results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or

interactions of one or more of the ingredients. Accordingly, in the context of the present invention, the terms "pharmaceutical agent" and "pharmaceutical composition" refer to any product made by admixing a molecule or compound of the present invention and a pharmaceutically or physiologically acceptable carrier.

[0041] The term "active ingredient" herein refers to a substance in an agent or composition that is biologically or physiologically active. Particularly, in the context of a pharmaceutical agent or composition, the term "active ingredient" refers to a component substance that shows an objective pharmacological effect. For example, in case of pharmaceutical agents or compositions for use in the treatment or prevention of cancer, active ingredients in the agents or compositions may lead to at least one biological or physiologically action on cancer cells and/or tissues directly or indirectly. Preferably, such action may include reducing or inhibiting cancer cell growth, damaging or killing cancer cells and/or tissues, and so on. Typically, indirect effect of active ingredients is inductions of CTLs that can recognize or kill cancer cells. Before being formulated, the "active ingredient" may also be referred to as "bulk", "drug substance" or "technical product".

[0042] The phrase "pharmaceutically acceptable carrier" or "physiologically acceptable carrier", as used herein, means a pharmaceutically or physiologically acceptable material, composition, substance or vehicle, including, but are not limited to, a liquid or solid filler, diluent, excipient, solvent and encapsulating material.

[0043] Some pharmaceutical agents or compositions of the present invention find particular use as vaccines. In the context of the present invention, the term "vaccine" (also referred to as an "immunogenic composition") refers to an agent or composition that has the function to improve, enhance and/or induce anti-tumor immunity upon inoculation into animals.

[0044] Unless otherwise defined, the term "cancer" refers to cancers or tumors that overexpress the SEMA5B gene, examples of which include, but are not limited to, esophageal cancer, NSCLC, RCC and SCLC.

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/cancer cells, virus-infected cells) and inducing the death of such cells.

[0045] Unless otherwise defined, the term "HLA-A24", as used herein, representatively refers to the subtypes, examples of which include, but are not limited to, HLA-A*2401, HLA-A*2402, HLA-A*2403, HLA-A*2404, HLA-A*2407, HLA-A*2408, HLA-A*2420, HLA-A*2425 and HLA-A*2488.

Unless otherwise defined, the term "kit" as used herein, is used in reference to a combination of reagents and other materials. It is contemplated herein that the kit may

include microarray, chip, marker, and so on. It is not intended that the term "kit" be limited to a particular combination of reagents and/or materials.

[0046] As used herein, in the context of a subject or patient, the phrase "subject's (or patient's) HLA antigen is HLA A24 " refers to that the subject or patient homozygously or heterozygously possess HLA-A24 antigen gene, and HLA-A24 antigen is expressed in cells of the subject or patient as an HLA antigen.

[0047] To the extent that the methods and compositions of the present invention find utility in the context of the "treatment" of cancer, a treatment is deemed "efficacious" if it leads to clinical benefit such as decrease in size, prevalence, or metastatic potential of cancer in a subject, prolongation of survival time, suppression of metastatic or postoperative recurrence and so on. When the treatment is applied prophylactically, "efficacious" means that it retards or prevents cancer from forming or prevents or alleviates a clinical symptom of cancer. Efficaciousness is determined in association with any known method for diagnosing or treating the particular tumor type.

[0048] To the extent that the methods and compositions of the present invention find utility in the context of the "prevention" and "prophylaxis" of cancer, such terms are interchangeably used herein to refer to any activity that 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 can 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.

[0049] In the context of the present invention, the treatment and/or prophylaxis of cancer and/or the prevention of metastatic or postoperative recurrence thereof include any activity that leads to the following events, such as the surgical removal of cancer cells, the inhibition of the growth of cancerous cells, the involution or regression of a tumor, the induction of remission and suppression of occurrence of cancer, the tumor regression, and the reduction or inhibition of metastasis, the suppression of post operative recurrence of cancer, and prolongation of survival time. Effective treatment 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 includes 10%, 20%, 30% or more reduction, or stable disease.

[0050] In the context of the present invention, the term "antibody" refers to immunoglobulins and fragments thereof that are specifically reactive to a designated protein or peptide thereof. An antibody can include human antibodies, primatized antibodies, chimeric antibodies, bispecific antibodies, humanized antibodies, antibodies fused to other proteins or radiolabels, and antibody fragments. Furthermore, an antibody herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity. An "antibody" indicates all classes (e.g., IgA, IgD, IgE, IgG and IgM).

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.

[0051] II. Peptides:

Peptides of the present invention described in detail below may be referred to as "SEMA5B peptide(s)" or " SEMA5B polypeptide(s)".

[0052] To demonstrate that peptides derived from SEMA5B function as an antigen recognized by CTLs, peptides derived from SEMA5B (SEQ ID NO: 75) were analyzed to determine whether they were antigen epitopes restricted by HLA-A24 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-A24 binding peptides derived from SEMA5B were identified based on their binding affinities to HLA-A24. The following candidate peptides were identified: SEQ ID NOs: 2 to 69.

[0053] Of the above, the following peptides resulted in the successful establishment of CTLs after in vitro stimulation of T-cells by dendritic cells (DCs) pulsed (loaded) with these peptides:

SEMA5B-A24-9-512 (SEQ ID NO: 2), SEMA5B-A24-9-1010 (SEQ ID NO: 3), SEMA5B-A24-9-196 (SEQ ID NO: 4), SEMA5B-A24-9-723 (SEQ ID NO: 8), SEMA5B-A24-9-280 (SEQ ID NO: 9), SEMA5B-A24-9-293 (SEQ ID NO: 10), SEMA5B-A24-9-470 (SEQ ID NO: 13), SEMA5B-A24-9-558 (SEQ ID NO: 20), SEMA5B-A24-10-354 (SEQ ID NO: 40), SEMA5B-A24-10-290 (SEQ ID NO: 41), SEMA5B-A24-10-1044 (SEQ ID NO: 47), and SEMA5B-A24-10-489 (SEQ ID NO: 54).

[0054] The established CTLs noted above showed potent specific CTL activity against target cells pulsed with respective peptides. These results demonstrate that SEMA5B is an antigen recognized by CTLs and that the above peptides are epitope peptides of

SEMA5B restricted by HLA-A24; therefore, such peptides may be effective as target antigens for cytotoxicity by CTLs.

[0055] Since the SEMA5B gene is over-expressed in cancer cells and tissues, including for example those of esophageal cancer, NSCLC, RCC and SCLC, and not expressed in most normal organs, it represents a good target for immunotherapy. Thus, the present invention provides nonapeptides (peptides composed of nine amino acid residues) and decapeptides (peptides composed of ten amino acid residues) corresponding to CTL-recognized epitopes from SEMA5B. Alternatively, the present invention provides isolated peptides which can induce CTLs, wherein the peptide is composed of an immunologically active fragment of SEMA5B. In some embodiments, the present invention provides peptides including an amino acid sequence selected from among SEQ ID NOs: 2 to 69, more preferably peptides including an amino acid sequence selected from among SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54. In preferred embodiments, the peptides of the present invention are nonapeptides or decapeptides including an amino acid sequence selected from among SEQ ID NOs: 2 to 69, particularly an amino acid sequence selected from among SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54. The preferred examples of the peptides of the present invention include peptides consisting of an amino acid sequence selected from among SEQ ID NOs: 2 to 69.

[0056] Generally, software programs now available, for example, on the Internet, such as those described in Parker KC et al., J Immunol 1994, 152(1): 163-75 and Nielsen M et al., Protein Sci 2003; 12: 1007-17 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 Parker KC et al., J Immunol 1994, 152(1): 163-75, Kuzushima K et al., Blood 2001, 98(6): 1872-81, Larsen MV et al. BMC Bioinformatics. 2007; 8: 424, Buus S et al. Tissue Antigens., 62:378-84, 2003, Nielsen M et al., Protein Sci 2003; 12: 1007-17, and Nielsen M et al. PLoS ONE 2007; 2: e796, which are summarized in, e.g., Lafuente EM et al., Current Pharmaceutical Design, 2009, 15, 3209-3220. Methods for determining binding affinity are described, for example, in the Journal of Immunological Methods(1995, 185: 181-190) and Protein Science (2000, 9: 1838-1846). Therefore, one of skill in the art can use such software programs to select those fragments derived from SEMA5B that have high binding affinity with HLA antigens. Accordingly, the present invention encompasses peptides composed of any fragments derived from SEMA5B, which would be determined to bind with HLA antigens by such known programs. Furthermore, such peptides may include the peptide consisting of the full length of SEMA5B sequence.

[0057] The peptides of the present invention, particularly the nonapeptides and decapeptides of the present invention, may be flanked with additional amino acid residues, so long

as the resulting peptide retains its CTL inducibility. The particular additional amino acid residues may be composed of any kind of amino acids, so long as they do not impair the CTL inducibility of the original peptide. Thus, the present invention encompasses peptides having CTL inducibility, in particular peptides derived from SEMA5B. Such peptides are, for example, less than about 40 amino acids, often less than about 20 amino acids, and usually less than about 15 amino acids.

[0058] It is generally known that the modification of one, two, several or more amino acids in a peptide do not influence the function of the peptide, and in some cases even enhance the desired function of the original peptide. In fact, modified peptides (i.e., peptides composed of an amino acid sequence in which 1, 2 or several amino acid residues have been modified (i.e., substituted, added, deleted and/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 have both CTL inducibility and an amino acid sequence selected from among SEQ ID NOs: 2 to 69, in which one, two or even more amino acids are added and/or substituted. In other words, the peptides of the present invention have both CTL inducibility and an amino acid sequence in which on, two or several amino acid(s) are substituted, deleted, inserted and/or added in the amino acid sequence selected from among SEQ ID NOs: 2 to 69, preferably in the amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54, provided the modified peptides retain the CTL inducibility of the original peptide.

[0059] Those of skill in the art will recognize that individual modifications (i.e., deletions, insertions, additions and/or substitutions) to an amino acid sequence that alter a single amino acid or a small percentage of the overall amino acid sequence 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 protein with similar functions to the original protein. Conservative substitution tables providing functionally similar amino acids are well known in the art. Examples of amino acid side-chains 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) Asparagine (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).

[0060] 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 may include non-conservative modifications, so long as the resulting modified peptide retains the requisite CTL inducibility of the original unmodified peptide. Furthermore, the modified peptides should not exclude CTL inducible peptides derived from polymorphic variants, interspecies homologues, and alleles of SEMA5B.

[0061] Amino acid residues may be inserted, substituted and/or added to the peptides of the present invention or, alternatively, amino acid residues may be deleted therefrom to achieve a higher binding affinity .To retain the requisite CTL inducibility, one of skill in the art preferably modifies (i.e., deletes, inserts, adds and/or substitutes) only a small number (for example, 1, 2 or several) or a small percentage of amino acids. Herein, the term "several" means 5 or fewer amino acids, for example, 4 or 3 or fewer. The percentage of amino acids to be modified may be, for example, 30% or less, preferably 20% or less, more preferably 15% of less, and even more preferably 10% or less, for example 1 to 5%.

[0062] When used in the context of cancer immunotherapy, the peptides of the present invention may be presented on the surface of a cell or exosome as a complex with an HLA antigen. Therefore, it is preferable to select peptides that not only induce CTLs but also 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 to the HLA antigen. In addition to peptides that are naturally displayed, since the regularity of the sequences of peptides displayed by binding to HLA antigens has already been known (J Immunol 1994, 152: 3913; Immunogenetics 1995, 41: 178; J Immunol 1994, 155: 4307), modifications based on such regularity may be introduced into the immunogenic peptides of the present invention.

[0063] For example, peptides possessing high HLA-A24 binding affinity tend to have the

second amino acid from the N-terminus substituted with phenylalanine, tyrosine, methionine or tryptophan. Likewise, peptides in which the C-terminal amino acid is substituted with phenylalanine, leucine, isoleucine, tryptophan or methionine tend to have high HLA-A24 binding affinity. Accordingly, it may be desirable to substitute the second amino acid from the N-terminus with phenylalanine, tyrosine, methionine or tryptophan, and/or the amino acid at the C-terminus with phenylalanine, leucine, isoleucine, tryptophan or methionine in order to increase the HLA-A24 binding affinity. Thus, peptides having an amino acid sequence selected from among SEQ ID NOs: 2 to 69, in which the second amino acid from the N-terminus of the amino acid sequence of the SEQ ID NO is substituted with phenylalanine, tyrosine, methionine or tryptophan, and/or in which the C-terminus of the amino acid sequence of the SEQ ID NO is substituted with phenylalanine, leucine, isoleucine, tryptophan or methionine are encompassed by the present invention. Also, the present invention encompasses the peptides including an amino acid sequence in which one, two or several amino acid are substituted, deleted, inserted and/or added in the SEQ ID NOs: 2 to 69, such peptides having one or both of the following characteristic of (a) the second amino acid from the N-terminus is phenylalanine, tyrosine, methionine or tryptophan; and (b) the C-terminal amino acid is phenylalanine, leucine, isoleucine, tryptophan or methionine. In preferred embodiments, the peptides of the present invention include an amino acid sequence in which the second amino acid from the N-terminus is substituted with phenylalanine, tyrosine, methionine or tryptophan, and/or the C-terminal amino acid is substituted with phenylalanine, leucine, isoleucine, tryptophan or methionine in the amino acid sequence of SEQ ID NOs: 2 to 69.

[0064] Substitutions can be introduced not only at the terminal amino acids but also at the positions of potential T cell receptor (TCR) recognition sites of peptides. Several studies have demonstrated that a peptide with amino acid substitutions may have equal to or better function than that of the original, for example, CAP1, $p53_{(264\text{-}272),}$ Her-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);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).

[0065] The present invention also contemplates the addition of 1, 2 or several amino acids can also be added to the N and/or C-terminus of the peptides of the present invention. Such modified peptides having high HLA antigen binding affinity and retaining CTL inducibility are also included in the present invention.

[0066] For example, the present invention provides an isolated peptide of less than 15, 14, 13, 12, 11, or 10 amino acids in length, which has CTL inducibility and comprises the amino acid sequence selected from the group consisting of:

(i) an amino acid sequence in which 1, 2 or several amino acid(s) are modified in the amino acid sequence selected from the group consisting of SEQ ID NOs: 2 to 39; and
(ii) the amino acid sequence of (i), wherein the amino acid sequence has one or both of the following characteristics:
(a) the second amino acid from the N-terminus of said SEQ ID NOs is or is modified to be an amino acid selected from the group consisting of phenylalanine, tyrosine, methionine and tryptophan, and
(b) the C-terminal amino acid of said SEQ ID NOs is or is modified to be an amino acid selected from the group consisting of phenylalanine, leucine, isoleucine, tryptophan and methionine.

[0067] The present invention also provides an isolated peptide of less than 15, 14, 13, 12, or 11 amino acids in length, which has CTL inducibility and comprises the amino acid sequence selected from the group consisting of:

(i') an amino acid sequence in which 1, 2 or several amino acid(s) are modified in the amino acid sequence selected from the group consisting of SEQ ID NOs: 40 to 69; and

(ii') the amino acid sequence of (i'), wherein the amino acid sequence has one or both of the following characteristics:

(a) the second amino acid from the N-terminus of said SEQ ID NOs is or is modified to be an amino acid selected from the group consisting of phenylalanine, tyrosine, methionine and tryptophan, and

(b) the C-terminal amino acid of said SEQ ID NOs is or is modified to be an amino acid selected from the group consisting of phenylalanine, leucine, isoleucine, tryptophan, and methionine.

These peptides are processed in an APC to present a peptide selected from the group consisting of (i) to (ii) and (i') to (ii') thereon, when these peptides are contacted with, or introduced in APC.

[0068] 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, negative side effects such as autoimmune disorders and/or allergic symptoms against specific substances may be induced. Therefore, it may be desirable 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 no peptide identical to or having only 1 or 2 amino acid differences as compared to the objective peptide exists in nature, 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.

[0069] 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 a peptide to induce a cytotoxic T lymphocyte (CTL) when presented on an antigen-presenting cell (APC). Further, "CTL inducibility" includes the ability of a peptide to induce CTL activation, CTL proliferation, promote lysis of target cells by a CTL, and to increase IFN-gamma production by a CTL.

[0070] Confirmation of CTL inducibility is accomplished by inducing APCs 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 of APCs with a test peptides, mixing the APCs with CD8 positive T cells to induce CTLs, and then measuring the IFN-gamma against the target cells produced and released by CTLs. 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, 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. Alternatively, the target cells may be radiolabeled with ^{51}Cr and such, and cytotoxic activity of CTLs may be calculated from radioactivity released from the target cells. Alternatively, CTL inducibility can be assessed by measuring IFN-gamma produced and released by CTLs in the presence of cells that carry immobilized peptides, and visualizing the inhibition zone on the media using anti-IFN-gamma monoclonal antibodies.

[0071] As a result of examining the CTL inducibility of the peptides as described above, it was discovered that nonapeptides or decapeptides selected from among those peptides having the amino acid sequences indicated by SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54 showed 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.

[0072] Furthermore, homology analysis results demonstrated that such peptides do not have significant homology with peptides derived from any other known human gene products. This lowers the possibility of unknown or undesired immune responses when used for immunotherapy. Therefore, also from this aspect, these peptides are useful for eliciting immunity against SEMA5B in cancer patients. Thus, peptides having an amino acid sequence selected from among SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54 are preferably encompassed by the present invention.

[0073] In addition to the above-described modifications, the peptides of the present invention can also be linked to other peptides, so long as the resulting linked peptide

retains the requisite CTL inducibility of the original peptide, and more preferably also retains the requisite HLA binding activity thereof. Examples of suitable "other" peptides include: the peptides of the present invention or the CTL-inducible peptides derived from other TAAs. The peptides of the present invention can be linked to one or more "other" peptides either directly or indirectly via a linker. The linkers between the peptides are well known in the art and include, for example AAY (P. M. Daftarian et al., J Trans Med 2007, 5:26), AAA, NKRK (SEQ ID NO: 81) (R. P. M. Sutmuller et al., J Immunol. 2000, 165: 7308-7315) or K (S. Ota et al., Can Res. 62, 1471-1476, K. S. Kawamura et al., J Immunol. 2002, 168: 5709-5715).

[0074] For example, peptides derived from non-SEMA5B tumor associated antigen can be also used subsequently or simultaneously to increase the immune response via HLA class I and/or class II. It is well-known in the art that cancer cells can express more than one tumor associated gene. Thus, it is within the scope of routine experimentation for one of ordinary skill in the art to determine whether a particular subject expresses additional tumor-associated genes, and then to include HLA class I and/or HLA class II binding peptides derived from expression products of such genes in SEMA5B compositions or vaccines of the present invention.

[0075] Examples of HLA class I and HLA class II binding peptides are known to those of ordinary skill in the art (for example, see Coulie, Stem Cells 13:393-403, 1995), and can be used in connection with the present invention in a like manner as those disclosed herein. One of ordinary skill in the art can prepare polypeptides including one or more SEMA5B peptides and one or more of the non-SEMA5B peptides, or nucleic acids encoding such polypeptides, using conventional molecular biology procedures.

[0076] The above described linked peptides are referred to herein as "polytopes", i.e., groups of two or more potentially immunogenic or immune response stimulating peptides which can be joined together in various arrangements (e.g., concatenated, overlapping). The polytope (or nucleic acid encoding the polytope) can be administered in accordance with a standard immunization protocol, e.g., to animals, to test the effectiveness of the polytope in stimulating, enhancing and/or provoking an immune response.

[0077] The peptides can be joined together directly or via the use of flanking sequences to form polytopes, and the use of polytopes as vaccines is well known in the art (see, e.g., Thomson et al., Proc. Natl. Acad. Sci USA 92(13):5845-5849, 1995; Gilbert et al., Nature Biotechnol. 15(12):1280-1284, 1997; Thomson et al., J Immunol. 157(2):822-826, 1996; Tarn et al., J Exp. Med. 171(l):299-306, 1990). Polytopes containing various numbers and combinations of epitopes can be prepared and tested for recognition by CTLs and for efficacy in increasing an immune response.

[0078] The peptides of the present invention may 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, for example: peptides, lipids, sugar and sugar chains, acetyl groups, natural and synthetic polymers, etc. The peptides may 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 may be performed to confer additional functions (e.g., targeting function, and delivery function) or to stabilize the peptide.

[0079] For example, to increase the in vivo stability of a peptide, it is known in the art to introduce D-amino acids, amino acid mimetics or unnatural amino acids; this concept can also be adapted to the peptides of the present invention. The stability of a peptide 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).

[0080] Moreover, as noted above, among the modified peptides in which are substituted, deleted, inserted and/or added by 1, 2 or several amino acid residues, those having same or higher activity as compared to original peptides can be screened for or selected. The present invention, therefore, also provides the method of screening for or selecting modified peptides having same or higher activity as compared to originals. An illustrative method includes the steps of:

a: modifying (i.e., substituting, deleting, inserting and/or adding) at least one amino acid residue of a peptide of the present invention,

b: determining the activity of the peptide modified in step a, and

c: selecting the peptide having same or higher activity as compared to the original peptide.

[0081] In preferred embodiments, the present invention provides a method of screening for a peptide having an ability to induce a CTL that has specific cytotoxic activity against a cell that presents a fragment derived from SEMA5B, wherein the method comprises the steps of:

(i) providing a candidate sequence consisting of an amino acid sequence modified by substituting, deleting, inserting and/or adding one, two or several amino acid residues to an original amino acid sequence, wherein the original amino acid sequence is selected from the group consisting of SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54;

(ii) selecting a candidate sequence that does not have substantial significant homology with the peptides derived from any known human gene products other than SEMA5B;

(iii) contacting a peptide consisting of the candidate sequence selected in step (ii)

with an antigen presenting cell;

(iv) contacting the antigen presenting cell of step (iii) with a CD8 positive T cell; and

(v) identifying the peptide of which CTL inducibility is same to or higher than a peptide consisting of the original amino acid sequence, or identifying the peptide having the CTL inducibility that is the same as or higher than the peptide consisting of the original amino acid sequence.

Herein, the activity to be assayed may include MHC binding activity, APC or CTL inducibility and cytotoxic activity. Preferably, the activity of the peptide is CTL inducibility.

[0082] III. Preparation of SEMA5B 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. The peptides of the present invention can be synthesized individually or as longer polypeptides including 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.

[0083] The peptides of the present invention may contain modifications, such as glycosylation, side chain oxidation, or phosphorylation, provided the modifications do not destroy the biological activity of the original peptide. Other illustrative modifications include incorporation of D-amino acids or other amino acid mimetics that can be used, for example, to increase the serum half life of the peptides.

[0084] Peptides of the present invention can be obtained through chemical synthesis based on the selected amino acid sequence. For example, conventional peptide synthesis methods that can be adopted for the synthesis include:

(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.

[0085] Alternatively, the peptides of the present invention can be obtained adopting any known genetic engineering method 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 polynu-

cleotide 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. Such vectors and host cells are also provided by the present invention. 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.

[0086] IV. Polynucleotides:

The present invention also provides polynucleotides that encode any of the afore-mentioned peptides of the present invention. These include polynucleotides derived from the natural occurring SEMA5B gene (GenBank Accession No. NM_001031702, NM_001256346, NM_001256347 or NM_001256348 (SEQ ID NO: 74, 76, 77 or 79)) 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 in the art 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.

[0087] The polynucleotides of the present invention can be composed of DNA, RNA, and derivatives thereof. As is well known in the art, a DNA is suitably composed of bases such as A, T, C, and G, and T is replaced by U in an RNA. One of skill in the art will recognize that non-naturally occurring bases may be included in polynucleotides, as well.

[0088] The polynucleotides of the present invention can encode multiple peptides of the present invention with or without intervening amino acid sequences. 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, a polynucleotide of the present invention can include any additional sequences to the coding sequence encoding a peptide of the present invention. For example, a polynucleotide of the present invention 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.

[0089] Both recombinant and chemical synthesis techniques can be used to produce the polynucleotides of the present invention. For example, the polynucleotides of the present invention can be produced by insertion into an appropriate vector, which can be expressed when transfected into a competent cell. Alternatively, the polynucleotides of the present invention 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, the polynucleotides of the present invention 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.

[0090] V. Exosomes:

The present invention further provides intracellular vesicles, called exosomes, that present complexes formed between the peptides of the present invention and HLA antigens on their surface. Exosomes can be prepared, for example, using the methods detailed in Japanese Patent Application JPH 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 the present invention can be inoculated as vaccines, in a fashion similar to the peptides of the present invention.

[0091] 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 population, HLA-A24, particularly HLA-A*2402, is prevalent and therefore would be appropriate for treatment of Japanese patients. The use of the HLA-A24 type that are highly expressed among the Japanese and Caucasian is favorable for obtaining effective results, and subtypes such as HLA-A*2402 also find 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, deletion and/or addition of 1, 2, or several amino acids can be performed based on the amino acid sequence of the naturally occurring SEMA5B partial peptide.

[0092] When using the HLA-A24 type of HLA antigen for the exosome of the present invention, peptides having an amino acid sequence selected from among SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54 have particular utility.

In some embodiments, the exosomes of the present invention present a complex of a peptide of the present invention and HLA-A24 antigen on their surface.

[0093] VI. Antigen-Presenting Cells (APCs):

The present invention also provides isolated antigen-presenting cells (APCs) that present complexes formed between HLA antigens and the peptides of the present invention on its surface. The APCs 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, exosomes, or CTLs of the present invention.

[0094] 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 DCs are representative APCs having the strongest CTL inducing action among APCs, DCs are suitable for the APCs of the present invention.

[0095] For example, the APCs of the present invention can be obtained by inducing DCs from peripheral blood monocytes and then contacting (stimulating) them with the peptides of the present invention in vitro, ex vivo or in vivo. When the peptides of the present invention are administered to a subject, APCs that present the peptides of the present invention are induced in the body of the subject. Therefore, the APCs of the present invention can be obtained by collecting the APCs from the subject after administering the peptides of the present invention to the subject. Alternatively, the APCs of the present invention can be obtained by contacting APCs collected from a subject with a peptide of the present invention.

[0096] The APCs of the present invention can be administered to a subject for inducing immune response against cancer in the subject by themselves or in combination with other drugs including the peptides, exosomes or CTLs of the present invention. For example, the ex vivo administration can include steps of:

a: collecting APCs from a first subject,

b: contacting the APCs of step a, with a peptide of the present invention, and

c: administering the APCs of step b to a second subject.

[0097] The first subject and the second subject can be the same individual, or may be different individuals. The APCs obtained by step b can be formulated and administered a vaccine for the treatment and/or prevention of cancer, such as esophageal cancer, NSCLC, RCC and SCLC, but not limited thereto.

[0098] In the context of the present invention, one may utilize the peptides of the present invention for manufacturing a pharmaceutical composition capable of inducing an antigen-presenting cell. The present invention also provides a method or process for manufacturing a pharmaceutical composition for inducing an antigen-presenting cell wherein the method includes the step of admixing or formulating the peptide of the present invention with a pharmaceutically acceptable carrier.

The present invention also provides for the use of the peptides of the present invention for inducing antigen-presenting cells.

[0099] According to an aspect of the present invention, the APCs of the present invention have CTL inducibility. In the context of the APCs, the phrase "CTL inducibility" refers to the ability of an APC to induce a CTL when contacted with a CD8 positive T cell. Further, "CTL inducibility" includes the ability of an APC to induce CTL activation, CTL proliferation, promote lysis of a target cell by a CTL, and to increase IFN-gamma production by a CTL. Such APCs having CTL inducibility can be prepared by a method that includes the step of transferring a polynucleotide encoding a peptide of the present invention to APCs in vitro as well as the method mentioned above. The introduced gene can be in the form of DNA or RNA. 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 partial peptides. Alternatively, APCs of the present invention can be prepared by a method which includes the step of simply contacting APCs with a peptide of the present invention.

[0100] In some embodiments, the APCs of the present invention present complexes of HLA-A24 antigen and a peptide of the present invention on their surface.

[0101] VII. Cytotoxic T Lymphocytes (CTLs):

A CTL induced against any one of the peptides of the present invention strengthens the immune response targeting cancer cells in vivo and thus can be used as vaccines, in a fashion similar to the peptides per se. Thus, the present invention provides isolated CTLs that are specifically induced or activated by any one of the peptides of the present invention.

[0102] Such CTLs can be obtained by (1) administering the peptide(s) of the present invention to a subject, (2) contacting (stimulating) subject-derived APCs, and CD8 positive T cells, or peripheral blood mononuclear leukocytes in vitro with the peptide(s) of the present invention, (3) contacting CD8 positive T cells or peripheral blood mononuclear leukocytes in vitro with the APCs or exosomes presenting a complex of an HLA antigen and the peptide on its surface or (4) introducing into a CD8 positive T cell a polynucleotide encoding both of T cell receptor (TCR) subunits or polynucleotides encoding each of TCR subunits, wherein the TCR formed by such subunits can bind a complex of a peptide of the present invention and HLA antigen on

a cell surface. Such APCs or exosomes can be prepared by the methods described above. Details of the method of (4) are described below in section "VIII. T cell receptor (TCR)".

[0103] The CTLs of the present 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, APCs or exosomes of the present invention for the purpose of regulating effects. The obtained CTLs act specifically against target cells presenting the peptides of the present invention, for example, the same peptides used for induction. The target cells can be cells that endogenously express SEMA5B, such as cancer cells, or cells that are transfected with the SEMA5B gene; and cells that present a peptide of the present invention on the cell surface due to stimulation by the peptide can also serve as targets of activated CTL attack.

[0104] In some embodiments, the CTLs of the present invention recognize cells presenting complexes of HLA-A24 antigen and a peptide of the present invention. In the context of the CTL, the phrase "recognize a cell" refers to binding a complex of HLA-A24 antigen and a peptide of the present invention on the cell surface via its TCR and showing specific cytotoxic activity against the cell. Herein, "specific cytotoxic activity" refers to showing cytotoxic activity against the cell presenting a complex of HLA-A24 antigen and a peptide of the present invention but not other cells. Accordingly, the CTLs that shows specific cytotoxic activity against a cell presenting a peptide of the present invention are included in the present invention.

[0105] VIII. T cell Receptor (TCR)

The present invention also provides a composition that include one or more polynucleotides encoding both of TCR subunits or polynucleotides encoding each of TCR subunits, wherein the TCR formed by such subunits can bind to a complex of an HLA antigen and a peptide of the present invention on a cell surface. Methods of using the same as also contemplated. Such TCR subunits have the ability to form TCRs that confer specificity to T cells against tumor cells expressing SEMA5B. By using the known methods in the art, the polynucleotides encoding each of alpha- and beta-chains of the TCR subunits of the CTL induced with one or more peptides of the present invention can be identified (WO2007/032255 and Morgan et al., J Immunol, 171, 3288 (2003)). For example, the PCR method is preferred to analyze the TCR. The PCR primers for the analysis can be, for example, 5'-R primers (5'-gtctaccaggcattcgcttcat-3') as 5' side primers (SEQ ID NO: 70) and 3-TRa-C primers (5'-tcagctggaccacagccgcagcgt-3') specific to TCR alpha chain C region (SEQ ID NO: 71), 3-TRb-C1 primers (5'-tcagaaatcctttctcttgac-3') specific to TCR beta chain C1 region (SEQ ID NO: 72) or 3-TRbeta-C2 primers (5'- ctagcctctggaatcctttctctt-3') specific to TCR beta chain C2 region (SEQ ID NO: 73) as 3' side primers, but not

limited thereto. The derivative TCRs can bind target cells presenting a peptide of the present invention with high avidity, and optionally mediate efficient killing of target cells presenting a peptide of the present invention in vivo and in vitro.

[0106] The polynucleotide encoding both of the TCR subunits or polynucleotides encoding each of the TCR subunits can be incorporated into suitable vectors, e.g., retroviral vectors. These vectors are well known in the art. The polynucleotides or the vectors including them usefully can be transferred into a T cell (e.g., CD8 positive T cell), for example, a T cell from a patient. Advantageously, the present 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.

[0107] Specific TCRs against peptides of the present invention should be capable of specifically recognizing a complex of a peptide of the present invention and HLA molecule, giving a T cell specific activity against a target cell presenting a complex of a peptide of the present invention and HLA antigen when the TCR is presented on the surface of the T cell. A specific recognition of the above complex may be confirmed by any known methods, preferred examples of which include HLA multimer staining analysis using HLA molecules and peptides of the present invention, and ELISPOT assay. By performing the ELISPOT assay, it can be confirmed that a T cell expressing the TCR on the cell surface recognizes a cell by the TCR, and that the signal is transmitted intracellularly. The confirmation that the above-mentioned complex can give a T cell cytotoxic activity when the complex exists on the T cell surface may also be carried out by a known method. A preferred method includes, for example, the determination of cytotoxic activity against an HLA positive target cell, such as chromium release assay.

[0108] Also, the present invention provides CTLs which are prepared by transduction with the polynucleotides encoding both of the TCR subunits or polynucleotides encoding each of the TCR subunits wherein the TCR formed by such TCR subunits can bind to the SEMA5B peptide, e.g., SEQ ID NOs: 2 to 69 in the context of HLA-A24.

[0109] 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 CTLs of the present invention can be used to form an immunogenic composition useful in either or both of treatment and the prevention of cancer in a patient in need of therapy or protection (See WO2006/031221 the contents of which are incorporated by reference herein).

[0110] IX. Pharmaceutical Compositions:

Since SEMA5B expression is specifically elevated in cancers, examples of which include, but are not necessarily limited to, esophageal cancer, NSCLC, RCC and SCLC

as compared to normal tissue, the peptides or polynucleotides of the present invention may be used to induce an immune response against a cancer or tumor cell and thus serve to treat and/or prevent cancer or primary cancer, and/or to prevent the metastatic or postoperative recurrence thereof. Thus, the present invention provides a pharmaceutical composition or agent formulated for the treatment and/or prophylaxis of cancer, and/or for the prevention of a metastatic or postoperative recurrence thereof, such composition or agent including at least one of the peptides or polynucleotides of the present invention as an active ingredient. Alternatively, the peptides of the present invention can be expressed on the surface of any of the foregoing exosomes or cells, such as APCs for the use as pharmaceutical compositions or agents. In addition, the aforementioned CTLs which target any one of the peptides of the present invention can also be used as the active ingredient of the pharmaceutical compositions or agents of the present invention.

[0111] Accordingly, the present invention provides agents or compositions including at least one active ingredient selected from among:

(a) a peptide of the present invention;

(b) a polynucleotide encoding such a peptide as disclosed herein in an expressible form;

(c) an APC or an exosome of the present invention; and

(d) a CTL of the present invention.

In the pharmaceutical composition or agent, such peptide, polynucleotide, APC, and CTL are present in a therapeutically or pharmaceutically effective amount.

[0112] The pharmaceutical compositions or agents of the present invention also find use as a vaccine. In the context of the present invention, the phrase "vaccine" (also referred to as an "immunogenic composition") refers to an agent or composition that has the function to improve, enhance and/or induce anti-tumor immunity upon inoculation into animals. In other words, the present invention provides the pharmaceutical agents or compositions for inducing an immune response against cancer in a subject.

[0113] The pharmaceutical compositions or agents of the present invention can be used to treat and/or prevent cancers, and/or prevent a metastatic or 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. In some embodiments, the pharmaceutical agents or compositions of the present invention can be formulated for the administration to a subject whose HLA antigen is HLA-A24.

[0114] In another embodiment, the present invention also provides the use of an active ingredient in manufacturing a pharmaceutical composition or agent for treating and/or

prevent cancer or tumor, and/or preventing a metastatic or post-operative recurrence thereof, said active ingredient selected from among:

(a) a peptide of the present invention;

(b) a polynucleotide encoding such a peptide as disclosed herein in an expressible form;

(c) an APC or an exosome presenting a peptide of the present invention on its surface; and

(d) a CTL of the present invention.

[0115] Alternatively, the present invention further provides an active ingredient for use in the treatment and/or prevention of cancers or tumors, and/or prevention of a metastatic or post-operative recurrence thereof, said active ingredient selected from among:

(a) a peptide of the present invention;

(b) a polynucleotide encoding such a peptide as disclosed herein in an expressible form;

(c) an APC or an exosome presenting a peptide of the present invention on its surface; and

(d) a CTL of the present invention.

[0116] Alternatively, the present invention further provides a method or process for the manufacture of a pharmaceutical composition or agent formulated for the treatment and/or prophylaxis of a cancer or tumor, and/or preventing a metastatic or post-operative recurrence thereof, 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 polynucleotide encoding such a peptide as disclosed herein in an expressible form;

(c) an APC or an exosome presenting a peptide of the present invention on its surface; and

(d) a CTL of the present invention.

[0117] In another embodiment, the present invention also provides a method or process for the manufacture of a pharmaceutical composition or agent formulated for the treatment and/or prophylaxis of a cancer or tumor, and/or preventing a metastatic or post-operative recurrence thereof, wherein the method or process includes the steps 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 polynucleotide encoding such a peptide as disclosed herein in an expressible form;

(c) an APC or an exosome presenting a peptide of the present invention on its surface; and

(d) a CTL T cell of the present invention.

[0118] In another embodiment, the present invention also provides a method for the treatment and/or prophylaxis of a cancer or tumor, and/or preventing a metastatic or post-operative recurrence thereof, wherein the method comprises the step of administering to a subject at least one active ingredient selected from among:

(a) a peptide of the present invention;

(b) a polynucleotide encoding such a peptide as disclosed herein in an expressible form;

(c) an APC or an exosome presenting a peptide of the present invention on its surface; and

(d) a CTL of the present invention.

[0119] According to the present invention, peptides having an amino acid sequence selected from among SEQ ID NOs: 2 to 69 shown to be HLA-A24 restricted epitope peptides or thus serve as candidates that can induce potent and specific immune response against cancer expressing HLA-A24 and SEMA5B in a subject. Therefore, the pharmaceutical compositions or agents which include any of these peptides with the amino acid sequence selected from among SEQ ID NOs: 2 to 69 are particularly suited for the administration to subjects whose HLA antigen is HLA-A24. The amount of the peptide in such agent or composition may be an amount that is effective in significantly inducing potent and specific immunological response in a subject carrying a cancer expressing SEMA5B.

The same applies to pharmaceutical compositions or agents that contain polynucleotides encoding any of these peptides (i.e., the polynucleotides of the present invention).

[0120] Cancers to be treated and/ or prevented by the pharmaceutical compositions or agents of the present invention are not limited and include all kinds of cancers in which SEMA5B is involved, examples of which include, but not limited to, esophageal cancer, NSCLC, RCC and SCLC.

[0121] The pharmaceutical compositions or agents of the present invention can contain in addition to the aforementioned active ingredients, other peptides that have the ability to induce CTLs against cancerous cells, other polynucleotides encoding the other peptides, other cells that present the other peptides, and the like. Examples of such "other" peptides having the ability to induce CTLs against cancerous cells include, but are not limited to, peptides derived from cancer specific antigens (e.g., identified TAAs).

[0122] If necessary, the pharmaceutical compositions or agents of the present invention can

optionally include other therapeutic substances as an additional active ingredient, so long as the substance does not inhibit the antitumoral effect of the active ingredient of the present invention, e.g., any of the peptides, polynucleotide, exosome, APC, CTL of the present invention. For example, formulations can include anti-inflammatory substances, 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 compositions. The amounts of medicament and pharmacologic composition depend, for example, on what type of pharmacologic composition(s) is/are used, the disease being treated, and the scheduling and routes of administration.

[0123] Those of skill in the art will recognize that, in addition to the ingredients particularly mentioned herein, the pharmaceutical compositions or agent of the present invention can include other substances conventional in the art having regard to the type of formulation in question.

[0124] In one embodiment of the present invention, the pharmaceutical compositions or agents of the present invention can be packaged 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 compositions or agents 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 composition or agent 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.

[0125] In addition to the container described above, a kit including a pharmaceutical composition or agent 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.

[0126] The pharmaceutical compositions or agents of the present invention can, if desired, be packaged 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.

[0127] (1) Pharmaceutical Compositions Containing the Peptides as the Active Ingredient:

The peptides of the present invention can be administered directly as a pharmaceutical composition or agent, or if necessary, may be formulated by conventional formulation methods. In the latter case, in addition to the peptides of the present invention, carriers, excipients, and such that are ordinarily used for drugs can be

included as appropriate without particular limitations. Examples of such carriers included but are not limited to, sterilized water, physiological saline, phosphate buffer, culture fluid and such. Furthermore, the pharmaceutical compositions or agents of the present invention can contain as necessary, stabilizers, suspensions, preservatives, surfactants and such. The pharmaceutical compositions or agents of the present invention can be used for anticancer purposes.

[0128] The peptides of the present invention can be prepared in combination, which includes two or more of peptides of the present invention, to induce CTLs in vivo. The peptides can be in 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. The peptides in the combination can be the same or different. By administering the peptides of the present invention, the peptides are presented in 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 (e.g., DCs) may be removed from a subject and then stimulated by the peptides of the present invention to obtain APCs that present any of the peptides of the present invention on their cell surface. These APCs can be re-administered to the subject to induce CTLs in the subject, and as a result, aggressiveness towards the tumor-associated endothelium can be increased.

[0129] The pharmaceutical compositions or agents for the treatment and/or prevention of cancer, that include any of peptides of the present invention as the active ingredient, can also include an adjuvant so that cellular immunity will be established effectively. Alternatively, the pharmaceutical compositions or agents of the present invention can be administered with other active ingredients, or can be administered by formulation into granules. An adjuvant refers to any compound, substance or composition 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, IFA (Incomplete Freund's adjuvant), CFA (Complete Freund's adjuvant), ISCOMatrix, GM-CSF, CpG, O/W emulsion and the like.

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.

[0130] In another embodiment, the peptides of the present invention may also be administered in the form of a pharmaceutically acceptable salt. Examples of preferred salts include, but are not limited to, salts with an alkali metal, salts with a metal, salts

with an organic base, salts with an amine, salts with an organic acid (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, malic acid, oxalic acid, benzoic acid, methanesulfonic acid and so on) and salts with an inorganic acid (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid , sulfuric acid, nitric acid, and so on). As used herein, the phrase "pharmaceutically acceptable salt" refers to those salts that retain the biological effectiveness and properties of the compound and which are obtained by reaction with inorganic or organic acids or bases such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

[0131] In some embodiments, the pharmaceutical compositions or agents of the present invention may further include a component that primes CTLs. Lipids have been identified as compositions capable of priming CTLs in vivo against viral antigens. For example, palmitic acid residues can be attached to the epsilon- and alpha-amino groups of a lysine residue and then linked to a peptide of the present invention. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant. As other examples of lipids, E. coli lipoproteins, such as tripalmitoyl-S-glycerylcysteinyl-seryl-serine (P3CSS) can be used to prime CTLs when covalently attached to an appropriate peptide (see, e.g., Deres et al., Nature 1989, 342: 561-4).

[0132] Examples of suitable methods of administration, include, but are not necessarily limited to, oral, intradermal, subcutaneous, intramuscular, intraosseous, peritoneal and intravenous injection, or such, and systemic administration or local administration to the vicinity of the targeted sites (i.e., direct injection). The administration can be performed by single administration or boosted by multiple administrations. A pharmaceutically or therapeutically effective amount of the peptide can be administered to a subject in need of treatment of cancer expressing SEMA5B. Alternatively, an amount of a peptide of the present invention sufficient to induce CTLs against cancer or tumor expressing SEMA5B can be administered to a subject carrying a cancer expressing SEMA5B. The dose of the peptides of the present 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, 0.01 mg to 100 mg, for example, 0.1 mg to 10 mg, for example, 0.5 mg to 5mg, and can be administered once in a few days to a few months, for example, once a week. One skilled in the art can readily determine suitable and optimal dosages.

[0133] (2) Pharmaceutical Compositions Containing Polynucleotides as the Active Ingredient:

The pharmaceutical compositions or agents of the present invention can also contain

nucleic acids encoding the peptide(s) 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 illustrative 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).

[0134] 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. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover et al., Nature 1991, 351: 456-60. A wide variety of other vectors useful for therapeutic administration or immunization e.g., adeno and adeno-associated virus vectors, retroviral vectors, Salmonella typhi vectors, detoxified anthrax toxin vectors, and the like, will be apparent. See, e.g., Shata et al., Mol Med Today 2000, 6: 66-71; Shedlock et al., J Leukoc Biol 2000, 68: 793-806; Hipp et al., In Vivo 2000, 14: 571-85.

[0135] Delivery of a polynucleotide into a patient can be direct, in which case the patient 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 patient. Theses two approaches are known, respectively, as in vivo and ex vivo gene therapies.

[0136] 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 that are applicable to the present invention are described by Ausubel et al. in Current Protocols in Molecular Biology (John Wiley & Sons, NY, 1993); and

Krieger in Gene Transfer and Expression, A Laboratory Manual (Stockton Press, NY, 1990).

[0137] Like administration of peptides, administration of polynucleotides may be performed by oral, intradermal, subcutaneous, intravenous, intramuscular, intraosseous, and/or peritoneal injection, or such, and via systemic administration or local administration to the vicinity of the targeted sites (i.e., direct injection). The administration can be performed by single administration or boosted by multiple administrations. A pharmaceutically or therapeutically effective amount of the polynucleotide can be administered to a subject in need of treatment of cancer expressing SEMA5B. Alternatively, an amount of the polynucleotides of the present invention sufficient to induce CTLs against cancer or tumor expressing SEMA5B can be administered to a subject carrying a cancer expressing SEMA5B. The dose of the polynucleotide in the suitable carrier or cells transformed with the polynucleotide encoding the peptides of the present 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, 0.01 mg to 100 mg, for example, 0.1 mg to 10 mg, for example, 0.5 mg to 5 mg, and can be administered once every a few days to once every few months, for example, once a week. One skilled in the art can readily determine suitable and optimal dosages.

[0138] X. Methods Using the Peptides, Exosomes, APCs and CTLs:

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

[0139] (1) Methods of inducing antigen-presenting cells (APCs)

The present invention provides methods of inducing APCs with CTL inducibility using the peptides or polynucleotides of the present invention.

The methods of the present invention include the step of contacting APCs with the peptides of the present invention in vitro, ex vivo or in vivo. For example, the method of contacting an APC with the peptide ex vivo can include steps of:

a: collecting APCs from a subject, and

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

[0140] The APCs are not limited to a particular kind of cells and include DCs, Langerhans cells, macrophages, B cells, and activated T cells, which are known to present pro-

teinaceous antigens on their cell surface so as to be recognized by lymphocytes. Preferably, DCs can be used since they have the strongest CTL inducibility among APCs. Any one of peptides of the present invention can be used by itself or in combination with one or more of other peptides of the present invention and/or one or more of CTL inducible peptides derived from TAAs other than SEMA5B.

[0141] On the other hand, when the peptides of the present invention are administered to a subject, APCs are contacted with the peptides in vivo, and consequently, APCs with CTL inducibility are induced in the body of the subject. Thus, the method of the present invention may include the step of administering a peptide of the present invention to a subject to induce APCs with CTL inducibility in the body of the subject. Similarly, when a polynucleotide of the present invention is administered to a subject in an expressible form, a peptide of the present invention is expressed and contacted with APCs in vivo, and consequently, APCs with CTL inducibility are induced in the body of the subject. Thus, the methods of the present invention may also include the step of administering a polynucleotide of the present invention to a subject to induce APCs with CTL inducibility in the body of the subject. The phrase "expressible form" is described above in section "IX. Pharmaceutical Compositions (2) Pharmaceutical compositions containing polynucleotides as the active ingredient".

[0142] Furthermore, the method of the present invention may include the step of introducing a polynucleotide of the present invention into an APC to induce an APC with CTL inducibility. For example, the method can include steps of:

a: collecting APCs from a subject, and

b: introducing a polynucleotide encoding a peptide of the present invention into an APC collected in step a.

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

[0143] Alternatively, the methods of the present invention may include the step of preparing an antigen-presenting cell (APC) that specifically induces CTL showing cytotoxic activity against SEMA5B, via one of the following steps:

(a) contacting an APC with a peptide of the present invention in vitro, ex vivo or in vivo; and

(b) introducing a polynucleotide encoding a peptide of the present invention into an APC.

[0144] Alternatively, the methods of the present invention may include the step of inducing an APC having CTL inducibility, such methods including a step selected from among:

(a) contacting an APC with a peptide of the present invention; and

(b) introducing the polynucleotide encoding a peptide of the present invention into an APC.

[0145] The methods of the present invention can be carried out in vitro, ex vivo or in vivo. Preferably, the methods of the present invention can be carried out in vitro or ex vivo. APCs used for induction of APCs having CTL inducibility can be preferably APCs expressing HLA-A24 antigen. Such APCs can be prepared by the methods well-known in the arts from peripheral blood mononuclear cells (PBMCs) obtained from a subject whose HLA antigen is HLA-A24. The APCs induced by the method of the present invention can be APCs that present a complex of a peptide of the present invention and HLA antigen (HLA A24 antigen) in its surface. When APCs induced by the method of the present invention are administered to a subject in order to induce immune responses against cancer in the subject, the subject is preferably the same one from whom APCs are derived. However, the subject may be a different one from the APC donor so long as the subject has the same HLA type with the APC donor.

[0146] In another embodiment, the present invention provide agents or compositions for use in inducing an APC having CTL inducibility, and such agents or compositions include one or more peptides or polynucleotides of the present invention.

In another embodiment, the present invention provides the use of a peptide of the present invention or the polynucleotide encoding the peptide in the manufacture of an agent or composition formulated for inducing APCs.

Alternatively, the present invention further provides a peptide of the present invention or the polypeptide encoding the peptide for use in inducing an APC having CTL inducibility.

[0147] (2) Method of Inducing CTLs:

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

The present invention also provides methods for inducing CTLs using a polynucleotide encoding both of TCR subunits or polynucleotides encoding each of TCR subunits, wherein the TCR formed by such subunits can recognize (bind to) a complex of a peptide of the present invention and an HLA antigen on a cell surface. Preferably, the methods for inducing CTLs may include at least one step selected from among:

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

b: contacting a CD8 positive T cell with an exosome that presents on its surface a complex of an HLA antigen and a peptide of the preset invention; and

c: introducing a polynucleotide encoding both of TCR subunits or polynucleotides encoding each of TCR subunits into a CD8 positive T cell, wherein the TCR formed by such subunits can recognize (bind to) a complex of a peptide of the present invention and an HLA antigen on a cell surface.

[0148] When the peptides, the polynucleotides, APCs, or exosomes of the present invention

are administered to a subject, CTLs are induced in the body of the subject, and the strength of immune responses targeting cancer cells expressing SEMA5B is enhanced. Thus, the methods of the present invention can include the step of administering the peptides, the polynucleotides, the APCs or exosomes of the present invention to a subject.

[0149] Alternatively, CTLs can be also induced by using them ex vivo or in vitro, and after inducing CTLs, the activated CTLs can be returned to the subject. For example, the method can include steps of:

a: collecting APCs from subject,

b: contacting the APCs of step a, with a peptide of the present invention, and

c: co-culturing the APCs of step b with CD8 positive T cells.

[0150] The APC to be co-cultured with the CD8 positive T cell in above step c can also be prepared by transferring a polynucleotide of the present invention into an APC as described above in section "VI. Antigen-presenting cells", although the present invention is not limited thereto and thus encompasses any APCs that effectively present on its surface a complex of an HLA antigen and a peptide of the present invention.

[0151] One may optionally utilize exosomes that present on the surface a complex of an HLA antigen and a peptide of the present invention instead of the aforementioned APCs. Namely, the present invention can includes the step of co-culturing exosomes presenting on its surface a complex of an HLA antigen and a peptide of the present invention and CD8 positive T cells. Such exosomes can be prepared by the methods described above in section "V. Exosomes". Suitable APCs and exosomes for the method of the present invention present a complex of a peptide of the present invention and HLA-A24 on its surface.

[0152] Furthermore, CTLs can be induced by introducing a polynucleotide encoding both of the TCR subunits or polynucleotides encoding each of the TCR subunits into CD8 positive T cell, wherein the TCR formed by such subunits can bind to a complex of a peptide of the present invention and an HLA antigen on a cell surface. Such transduction can be performed as described above in section "VIII. T cell receptor (TCR)".

[0153] The methods of the present invention can be carried out in vitro, ex vivo or in vivo. Preferably, the methods of the present invention can be carried out in vitro or ex vivo. CD8 positive T cells used for induction of CTLs can be prepared by well-known methods in the art from PBMCs obtained from a subject. In preferred embodiments, the donor for CD8 positive T cells can be a subject whose HLA antigen is HLA-A24. The CTLs induced by the methods of the present invention can be CTLs that can recognize cells presenting a complex of a peptide of the present invention and an HLA

antigen on its surface. Such CTLs can show specific cytotoxic activity against cells that present a peptide of the present invention on its surface, and therefore, can show specific cytotoxic activity against cells expressing SEMA5B (e.g., cancer cells). When CTLs induced by the method of the present invention are administered to a subject in order to induce immune responses against cancer in the subject, the subject is preferably the same one from whom CD8 positive T cells are derived. However, the subject may be a different one from the CD8 positive T cell donor so long as the subject has the same HLA type with the CD8 positive T cell donor.

[0154] In addition, the present invention provides a method or process for the manufacture of a pharmaceutical composition or agent that induces a CTL, wherein the method or process includes the step of admixing or formulating a peptide of the present invention with a pharmaceutically acceptable carrier.

[0155] In another embodiment, the present invention provides an agent or composition for inducing a CTL, wherein the agent or composition comprises one or more peptide(s), one or more polynucleotide(s), one or more APC(s), and/or one or more exosome(s) of the present invention.

In another embodiment, the present invention provides the use of the peptide, polynucleotide, APC or exosome of the present invention in the manufacture of an agent or composition formulated for inducing a CTL.

Alternatively, the present invention further provides the peptide, polynucleotide, APC or exosome of the present invention for use in inducing a CTL.

[0156] XI. Methods of Inducing Immune Response:

Moreover, the present invention provides methods of inducing immune responses against diseases related to SEMA5B. Diseases contemplated include cancer, examples of which include, but are not limited to, esophageal cancer, NSCLC, RCC and SCLC.

The methods of the present invention may include the step of administering an agent or composition containing any of the peptides of the present invention or polynucleotides encoding them. The inventive methods also contemplate the administration of exosomes or APCs presenting any of the peptides of the present invention. For details, see the item of "IX. Pharmaceutical Compositions", particularly the part describing the use of the pharmaceutical compositions of the present invention as vaccines. In addition, the exosomes and APCs that can be employed for the present methods for inducing immune response are described in detail under the items of "V. Exosomes", "VI. Antigen-presenting cells (APCs)", and (1) and (2) of "X. Methods Using the Peptides, Exosomes, APCs and CTLs", supra.

[0157] The present invention also provides a method or process for the manufacture of a pharmaceutical composition or agent that induces immune response against cancer, wherein the method may include the step of admixing or formulating a peptide of the

present invention with a pharmaceutically acceptable carrier.

[0158] Alternatively, the method of the present invention may include the step of administrating a vaccine or a pharmaceutical composition or agent of the present invention that contains:

(a) a peptide of the present invention;

(b) a polynucleotide encoding such a peptide as disclosed herein in an expressible form;

(c) an APC presenting a peptide of the present invention on its surface;

(d) an exosome presenting a peptide of the present invention on its surface; or

(e) a CTL of the present invention.

[0159] In the context of the present invention, a cancer over-expressing SEMA5B can be treated with these active ingredients. Examples of such cancer include, but are not limited to, esophageal cancer, NSCLC, RCC and SCLC. Accordingly, prior to the administration of the vaccines or pharmaceutical compositions or agent including any of aforementioned active ingredients, it is preferable to confirm whether the expression level of SEMA5B in cancerous cells or tissues collected from the subject to be treated is elevated as compared with normal cells or tissues collected from the same subject. Thus, in one embodiment, the present invention provides a method for treating cancer (over)expressing SEMA5B in a patient in need thereof, such method including the steps of:

i) determining the expression level of SEMA5B in a biological sample obtained from a subject with the cancer to be treated;

ii) comparing the expression level of SEMA5B with normal control; and

iii) administrating at least one component selected from among (a) to (e) described above to a subject with cancer over-expressing SEMA5B as compared with normal control.

[0160] Alternatively, the present invention provides a vaccine or pharmaceutical composition including at least one component selected from among (a) to (e) described above, to be administered to a subject having cancer over-expressing SEMA5B. In other words, the present invention further provides a method for identifying a subject to be treated with a peptide of the present invention, such method including the step of determining an expression level of SEMA5B in a subject-derived biological sample, wherein an increase of the expression level as compared to a normal control level of the gene indicates that the subject may have cancer which may be treated with a peptide of the present invention. The methods of treating cancer of the present invention will be described in more detail in below.

[0161] Any subject-derived cell or tissue can be used for the determination of the expression level of SEMA5B so long as it can include the transcription or translation product of

SEMA5B. Examples of suitable samples include, but are not limited to, bodily tissues and fluids, such as blood, sputum and urine. Preferably, the subject-derived cell or tissue sample contains a cell population including an epithelial cell, more preferably a cancerous epithelial cell or an epithelial cell derived from cancerous tissue. Further, if necessary, the cell may be purified from the obtained bodily tissues and fluids, and then used as the subjected-derived sample.

[0162] A subject to be treated by the method of the present invention is preferably a mammal. Illustrative mammals include, but are not limited to, e.g., human, non-human primate, mouse, rat, dog, cat, horse, and cow.

[0163] According to the present invention, the expression level of SEMA5B in a biological sample obtained from a subject may be determined. The expression level of SEMA5B can be determined at the transcription (nucleic acid) product level, using methods known in the art. For example, the mRNA of SEMA5B may be quantified using probes by hybridization methods (e.g., Northern hybridization). The detection may be carried out on a chip or an array. The use of an array is preferable for detecting the expression level of SEMA5B. Those skilled in the art can prepare such probes utilizing the sequence information of SEMA5B. For example, the cDNA of SEMA5B may be used as the probes. If necessary, the probes may be labeled with a suitable label, such as dyes, fluorescent substances and isotopes, and the expression level of SEMA5B may be detected as the intensity of the hybridized labels.

[0164] Furthermore, the transcription product of SEMA5B may be quantified using primers by amplification-based detection methods (e.g., RT-PCR). Such primers may be prepared based on the available sequence information of SEMA5B.

[0165] Specifically, a probe or primer used for the present method hybridizes under stringent, moderately stringent, or low stringent conditions to the mRNA of SEMA5B. As used herein, the phrase "stringent (hybridization) conditions" refers to conditions under which a probe or primer will hybridize to its target sequence, but not to other sequences. Stringent conditions are sequence-dependent and will be different under different circumstances. Specific hybridization of longer sequences is observed at higher temperatures than shorter sequences. Generally, the temperature of a stringent condition is selected to be about 5 degree Centigrade lower than the thermal melting point (Tm) for a specific sequence at a defined ionic strength and pH. The Tm is the temperature (under a defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to their target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the

temperature is at least about 30 degree Centigrade for short probes or primers (e.g., 10 to 50 nucleotides) and at least about 60 degree Centigrade for longer probes or primers. Stringent conditions may also be achieved with the addition of destabilizing substances, such as formamide.

[0166] A probe or primer of the present invention is typically a substantially purified oligonucleotide. The oligonucleotide typically includes a region of nucleotide sequence that hybridizes under stringent conditions to at least about 2000, 1000, 500, 400, 350, 300, 250, 200, 150, 100, 50, or 25, consecutive sense strand nucleotide sequence of a nucleic acid including a SEMA5B sequence, or an anti-sense strand nucleotide sequence of a nucleic acid including a SEMA5B sequence, or of a naturally occurring mutant of these sequences. In particular, for example, in a preferred embodiment, an oligonucleotide having 5-50 b(bases) in length can be used as a primer for amplifying the genes, to be detected. More preferably, mRNA or cDNA of a SEMA5B gene can be detected with oligonucleotide probe or primer of a specific size, generally 15- 30b in length. The size may range from at least 10 nucleotides, at least 12 nucleotides, at least 15 nucleotides, at least 20 nucleotides, at least 25 nucleotides, at least 30 nucleotides and the probes and primers may range in size from 5-10 nucleotides, 10-15 nucleotides, 15-20 nucleotides, 20-25 nucleotides and 25-30 nucleotides. In preferred embodiments, length of the oligonucleotide probe or primer can be selected from 15-25 nucleotides. Assay procedures, devices, or reagents for the detection of gene by using such oligonucleotide probe or primer are well known (e.g. oligonucleotide microarray or PCR). In these assays, probes or primers can also include tag or linker sequences. Further, probes or primers can be modified with detectable label or affinity ligand to be captured. Alternatively, in hybridization based detection procedures, a polynucleotide having a few hundreds (e.g., about 100-200) bases to a few kilo (e.g., about 1000-2000) bases in length can also be used for a probe (e.g., northern blotting assay or cDNA microarray analysis).

[0167] Alternatively, the translation product of SEMA5B may be detected for the identification of a subject to be treated by the method of the present invention. For example, the quantity of SEMA5B protein (SEQ ID NO: 75, 78 or 80) may be determined. Methods for determining the quantity of the SEMA5B protein as the translation product include immunoassay methods using an antibody specifically recognizing the SEMA5B protein. The antibody may be monoclonal or polyclonal. Furthermore, any fragment or modification (e.g., chimeric antibody, scFv, Fab, $F(ab')_2$, Fv, etc.) of the antibody may be used for the detection, so long as the fragment or modified antibody retains the binding ability to the SEMA5B protein. Methods to prepare these kinds of antibodies are well known in the art, and any method may be employed to prepare such antibodies and equivalents thereof.

[0168] As another method to detect the expression level of SEMA5B based on its translation product, the intensity of staining may be measured via immunohistochemical analysis using an antibody against the SEMA5B protein. Namely, in this measurement, strong staining indicates increased presence/level of the SEMA5B protein and, at the same time, high expression level of SEMA5B.

[0169] The expression level of the SEMA5B gene in a subject-derived sample can be determined to be increased if the expression level increases from the control level (e.g., the expression level in normal cells) of the SEMA5B by, for example, 10%, 25%, or 50%; or increases to more than 1.1 fold, more than 1.5 fold, more than 2.0 fold, more than 5.0 fold, more than 10.0 fold, or more.

[0170] The control level may be determined at the same time as the cancer cells by using a sample(s) previously collected and stored from a healthy subject/subjects. In addition, normal cells obtained from non-cancerous regions of an organ that has the cancer to be treated may be used as normal control. Alternatively, the control level may be determined by a statistical method based on the results obtained by analyzing previously determined expression level(s) of SEMA5B in samples from subjects whose disease states are known. Furthermore, the control level can be derived from a database of expression patterns from previously tested cells. Moreover, according to an aspect of the present invention, the expression level of SEMA5B in a biological sample may be compared to multiple control levels, which are determined from multiple reference samples. It is preferred to use a control level determined from a reference sample derived from a tissue type similar to that of the subject-derived biological sample. Moreover, it is preferred to use the standard value of the expression levels of SEMA5B gene in a population with a known disease state. The standard value may be obtained by any method known in the art. For example, a range of mean +/- 2 S.D. or mean +/- 3 S.D. may be used as the standard value.

[0171] In the context of the present invention, a control level determined from a biological sample that is known to be non-cancerous is referred to as a "normal control level". On the other hand, if the control level is determined from a cancerous biological sample, it is referred to as a "cancerous control level". Difference between a sample expression level and a control level can be normalized to the expression level of control nucleic acids, e.g., housekeeping genes, whose expression levels are known not to differ depending on the cancerous or non-cancerous state of the cell. Exemplary control genes include, but are not limited to, beta-actin, glyceraldehyde 3 phosphate dehydrogenase, and ribosomal protein P1.

[0172] When the expression level of SEMA5B is increased as compared to the normal control level, the subject may be identified as a subject with cancer to be treated by administration of a pharmaceutical composition or agent of the present invention.

[0173] The present invention also provides a method of selecting a subject for cancer treatment using aforementioned pharmaceutical compositions or agents of the present invention, such method including the steps of:

a) determining the expression level of SEMA5B in biological sample(s) obtained from a subject with cancer;

b) comparing the expression level of SEMA5B determined in step a) with a normal control level; and

c) selecting the subject for cancer treatment by the pharmaceutical compositions or agents of the present invention, if the expression level of SEMA5B is increased as compared to the normal control level.

[0174] The present invention also provides a diagnostic kit for identifying or determining a subject who is suffering from or at risk of developing a cancer that can be treated with a pharmaceutical composition or agent of the present invention, which may also be useful in assessing and/or monitoring the efficacy or applicability of a cancer immunotherapy. Preferably, the cancer includes, but is not limited to, esophageal cancer, NSCLC, RCC and SCLC. More particularly, the kit preferably includes at least one reagent for detecting the expression level of the SEMA5B in a subject-derived sample, which reagent may be selected from the group consisting of:

(a) a reagent for detecting the SEMA5B mRNA;

(b) a reagent for detecting the SEMA5B protein; and

(c) a reagent for detecting the biological activity of the SEMA5B protein.

[0175] Examples of reagents suitable for detecting the SEMA5B mRNA include nucleic acids that specifically bind to or identify the SEMA5B mRNA, such as oligonucleotides that have a complementary sequence to a portion of the SEMA5B mRNA. These kinds of oligonucleotides are exemplified by primers and probes that are specific to the SEMA5B mRNA. These kinds of oligonucleotides may be prepared based on methods well known in the art. If needed, the reagent for detecting the SEMA5B mRNA may be immobilized on a solid matrix. Moreover, more than one reagent for detecting the SEMA5B mRNA may be included in the kit.

[0176] On the other hand, examples reagents suitable for detecting the SEMA5B protein may include antibodies to the SEMA5B protein. The antibody may be monoclonal or polyclonal. Furthermore, any fragment or modification (e.g., chimeric antibody, scFv, Fab, $F(ab')_2$, Fv, etc.) of the antibody may be used as the reagent, so long as the fragment or modified antibody retains the binding ability to the SEMA5B protein. Methods to prepare these kinds of antibodies for the detection of SEMA5B protein are well known in the art, and any method may be employed in the present invention to prepare such antibodies and equivalents thereof. Furthermore, the antibody may be labeled with signal generating molecules via direct linkage or an indirect labeling

technique. Labels and methods for labeling antibodies and detecting the binding of the antibodies to their targets are well known in the art, and any labels and methods may be employed for the present invention. Moreover, more than one reagent for detecting the SEMA5B protein may be included in the kit.

[0177] The kit may contain more than one of the aforementioned reagents. The kit can further include a solid matrix and reagent for binding a probe against a SEMA5B mRNA or antibody against a SEMA5B protein, a medium and container for culturing cells, positive and negative control reagents, and a secondary antibody for detecting an antibody against a SEMA5B protein. For example, tissue samples obtained from subjects without cancer may serve as useful control reagents. A kit of the present invention may further include other materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts (e.g., written, tape, CD-ROM, etc.) with instructions for use. These reagents and such may be retained in a container with a label. Suitable containers include bottles, vials, and test tubes. The containers may be formed from a variety of materials, such as glass or plastic.

[0178] In one embodiment, when the reagent is a probe against the SEMA5B mRNA, the reagent may be immobilized on a solid matrix, such as a porous strip, to form at least one detection site. The measurement or detection region of the porous strip may include a plurality of sites, each containing a nucleic acid (probe). A test strip may also contain sites for negative and/or positive controls. Alternatively, control sites may be located on a strip separated from the test strip. Optionally, the different detection sites may contain different amounts of immobilized nucleic acids, i.e., a higher amount in the first detection site and lesser amounts in subsequent sites. Upon the addition of a test sample, the number of sites displaying a detectable signal provides a quantitative indication of the amount of SEMA5B mRNA present in the sample. The detection sites may be configured in any suitably detectable shape and are typically in the shape of a bar or dot spanning the width of a test strip.

[0179] The kit of the present invention may further include a positive control sample or SEMA5B standard sample. The positive control sample of the present invention may be prepared by collecting SEMA5B positive samples and then assaying their SEMA5B levels. Alternatively, a purified SEMA5B protein or polynucleotide may be added to cells that do not express SEMA5B to form the positive sample or the SEMA5B standard sample. In the present invention, purified SEMA5B may be a recombinant protein. The SEMA5B level of the positive control sample is, for example, more than the cut off value.

[0180] In one embodiment, the present invention further provides a diagnostic kit including one or more peptide of the present invention.

Cancer can be diagnosed by detecting antibodies against a peptide of the present invention in a subject-derived sample (e.g., blood, tissue) using a peptide of the present invention.

[0181] Diagnosis of cancer can be performed by determining the difference between the amount of anti-SEMA5B antibody and that in the corresponding control sample as describe above. The subject is suspected to be suffering from cancer, if a subject-derived sample (e.g., blood sample) contains antibodies against a peptide of the present invention and the quantity of the antibodies is determined to be more than the cut off value as compared to control level.

[0182] In another embodiment, a diagnostic kit of the present invention may include a peptide of the present invention and an HLA molecule binding thereto. The method for detecting antigen specific CTLs using antigenic peptides and HLA molecules has already been established (for example, Altman JD et al., Science. 1996, 274(5284): 94-6). Thus, the complex of a peptide of the present invention and the HLA molecule can be applied to the detection method to detect tumor antigen specific CTLs, thereby enabling earlier detection, recurrence and/or metastasis of cancer. Further, it can be employed for the selection of subjects applicable with the pharmaceuticals including a peptide of the present invention as an active ingredient, or the assessment of the treatment effect of the pharmaceuticals.

[0183] Particularly, according to the known method (see, for example, Altman JD et al., Science. 1996, 274(5284): 94-6), the oligomer complex, such as tetramer, of the radiolabeled HLA molecule and a peptide of the present invention can be prepared. With using the complex, the diagnosis can be done, for example, by quantifying the antigen-peptide specific CTLs in the peripheral blood lymphocytes derived from the subject suspected to be suffering from cancer.

[0184] The present invention further provides methods and diagnostic agents for evaluating immunological response of subject by using peptide epitopes as described herein. In one embodiment of the invention, HLA-A24 restricted peptides as described herein are used as reagents for evaluating or predicting an immune response of a subject. The immune response to be evaluated is induced by contacting an immunogen with immunocompetent cells in vitro or in vivo. In preferred embodiments, the immunocompetent cells for evaluating an immunological response, may be selected from among peripheral blood, peripheral blood lymphocyte (PBL), and peripheral blood mononuclear cell (PBMC). Methods for collecting or isolating such immunocompetent cells are well known in the arts. In some embodiments, any agent that may result in the production of antigen specific CTLs that recognize and bind to the peptide epitope (s) may be employed as the reagent. The peptide reagent need not be used as the immunogen. Assay systems that are used for such an analysis include relatively recent

technical developments such as tetramers, staining for intracellular lymphokines and interferon release assays, or ELISPOT assays. In a preferred embodiment, immunocompetent cells to be contacted with peptide reagent may be antigen presenting cells including dendritic cells.

[0185] For example, peptides of the present invention may be used in tetramer staining assays to assess peripheral blood mononuclear cells for the presence of antigen-specific CTLs following exposure to a tumor cell antigen or an immunogen. The HLA tetrameric complex may be used to directly visualize antigen specific CTLs (see, e. g., Ogg et al., Science 279 : 2103-2106, 1998 ; and Altman et al, Science 174 : 94-96, 1996) and determine the frequency of the antigen-specific CTL population in a sample of peripheral blood mononuclear cells. A tetramer reagent using a peptide of the present invention may be generated as described below.

[0186] A peptide that binds to an HLA molecule is refolded in the presence of the corresponding HLA heavy chain and beta 2- microglobulin to generate a trimolecular complex. In the complex, carboxyl terminal of the heavy chain is biotinylated at a site that was previously engineered into the protein. Then, streptavidin is added to the complex to form tetramer composed of the trimolecular complex and streptavidin. By means of fluorescently labeled streptavidin, the tetramer can be used to stain antigen-specific cells. The cells can then be identified, for example, by flow cytometry. Such an analysis may be used for diagnostic or prognostic purposes. Cells identified by the procedure can also be used for therapeutic purposes.

[0187] The present invention also provides reagents to evaluate immune recall responses (see, e. g., Bertoni et al, J. Clin. Invest. 100: 503-513, 1997 and Penna et al, J Exp. Med. 174: 1565-1570, 1991) including peptides of the present invention. For example, patient PBMC samples obtained from individuals with a cancer to be treated are analyzed for the presence of antigen-specific CTLs using specific peptides. A blood sample containing mononuclear cells can be evaluated by cultivating the PBMCs and stimulating the cells with a peptide of the present invention. After an appropriate cultivation period, the expanded cell population can be analyzed, for example, for CTL activity.

[0188] The peptides may be also used as reagents to evaluate the efficacy of a vaccine. PBMCs obtained from a patient vaccinated with an immunogen may be analyzed using, for example, either of the methods described above. The patient is HLA typed, and peptide epitope reagents that recognize the allele specific molecules present in that patient are selected for the analysis. The immunogenicity of the vaccine may be indicated by the presence of epitope-specific CTLs in the PBMC sample.

[0189] The peptides of the invention may be also used to make antibodies, using techniques well known in the art (see, e. g. CURRENT PROTOCOLS IN IMMUNOLOGY,

Wiley/Greene, NY ; and Antibodies A Laboratory Manual, Harlow and Lane, Cold Spring Harbor Laboratory Press, 1989), which may be useful as reagents to diagnose or monitor cancer. Such antibodies may include those that recognize a peptide in the context of an HLA molecule, i. e., antibodies that bind to a peptide-MHC complex.

[0190] The peptides and compositions of the present invention have a number of additional uses, some of which are described herein. For instance, the present invention provides a method for diagnosing or detecting a disorder characterized by quantity of a SEMA5B immunogenic polypeptide. These methods involve determining quantity of a SEMA5B peptide, or a complex of a SEMA5B peptide and an HLA class I molecule in a biological sample. The expression of a peptide or complex of the peptide and HLA class I molecule can be determined or detected by assaying with a binding partner for the peptide or complex. In a preferred embodiment, a binding partner for the peptide or complex is an antibody that recognizes and specifically binds to the peptide. The expression of SEMA5B in a biological sample, such as a tumor biopsy, can also be tested by standard PCR amplification protocols using SEMA5B primers. An example of tumor expression is presented herein and further disclosure of exemplary conditions and primers for SEMA5B amplification can be found in WO2003/27322.

[0191] Preferably, the diagnostic methods involve contacting a biological sample isolated from a subject with an agent specific for the SEMA5B peptide to detect the presence of the SEMA5B peptide in the biological sample. As used herein, "contacting" means placing the biological sample in sufficient proximity to the agent and under the appropriate conditions of, e. g., concentration, temperature, time, ionic strength, to allow the specific interaction between the agent and SEMA5B peptide that are present in the biological sample. In general, the conditions for contacting the agent with the biological sample are conditions known by those of ordinary skill in the art to facilitate a specific interaction between a molecule and its cognate (e. g., a protein and its receptor cognate, an antibody and its protein antigen cognate, a nucleic acid and its complementary sequence cognate) in a biological sample. Optimal conditions for facilitating a specific interaction between a molecule and its cognate are described in U. S. Patent No. 5,108,921, issued to Low et al.

[0192] The diagnostic method of the present invention can be performed in either or both of in vivo and in vitro. Accordingly, biological sample can be located in vivo or in vitro in the present invention. For example, the biological sample can be a tissue in vivo and the agent specific for the SEMA5B immunogenic polypeptide can be used to detect the presence of such molecules in the tissue. Alternatively, the biological sample can be collected or isolated in vitro (e. g., a blood sample, tumor biopsy, tissue extract). In a particularly preferred embodiment, the biological sample can be a cell- containing sample, more preferably a sample containing tumor cells collected from a subject to be

diagnosed or treated.

[0193] Alternatively, the diagnosis can be done, by a method which allows direct quantification of antigen- specific T cells by staining with Fluorescein-labelled HLA multimeric complexes (for example, Altman, J. D. et al., 1996, Science 274 : 94; Altman, J. D. et al., 1993, Proc. Natl. Acad. Sci. USA 90 : 10330 ;). Staining for intracellular lymphokines, and interferon-gamma release assays or ELISPOT assays also has been provided. Tetramer staining, intracellular lymphokine staining and ELISPOT assays all appear to be at least 10-fold more sensitive than more conventional assays (Murali-Krishna, K. et al., 1998, Immunity 8 : 177; Lalvani, A. et al., 1997, J. Exp. Med. 186 : 859; Dunbar, P. R. et al., 1998, Curr. Biol. 8 : 413 ;). Pentamers (e.g., US 2004-209295A), dextramers (e.g., WO 02/072631), and streptamers (e.g., Nature medicine 6. 631-637 (2002)) may also be used.

[0194] For instance, in some embodiments, the present invention provides a method for diagnosing or evaluating an immunological response of a subject administered at least one of SEMA5B peptides of the present invention, the method including the steps of:

(a) contacting an immunogen with immunocompetent cells under the condition suitable for induction of CTL specific to the immunogen;

(b) detecting or determining induction level of the CTL induced in step (a); and

(c) correlating the immunological response of the subject with the CTL induction level.

[0195] In the context of the present invention, the immunogen preferably includes at least one of (a) a SEMA5B peptide selected from among the amino acid sequences of SEQ ID NOs: 2 to 69, peptides having such amino acid sequences, and peptides having in which such amino acid sequences have been modified with 1, 2 or more amino acid substitution(s). In the meantime, conditions suitable of induction of immunogen specific CTL are well known in the art. For example, immunocompetent cells may be cultured in vitro under the presence of immunogen(s) to induce immunogen specific CTL. In order to induce immunogen specific CTLs, any stimulating factors may be added to the cell culture. For example, IL-2 is preferable stimulating factors for the CTL induction.

[0196] In some embodiments, the step of monitoring or evaluating immunological response of a subject to be treated with peptide cancer therapy may be performed before, during and/or after the treatment. In general, during a protocol of cancer therapy, immunogenic peptides are administered repeatedly to a subject to be treated. For example, immunogenic peptides may be administered every week for 3-10 weeks. Accordingly, the immunological response of the subject can be evaluated or monitored during the cancer therapy protocol. Alternatively, the step of evaluation or monitoring of immunological response to the cancer therapy may at the completion of the therapy

protocol.

[0197] According to the present invention, enhanced induction of immunogen specific CTL as compared with a control indicates that the subject to be evaluated or diagnosed immunologically responded to the immunogen(s) that has/have been administered. Suitable controls for evaluating the immunological response may include, for example, a CTL induction level when the immunocompetent cells are contacted with no peptide, or control peptide(s) having amino acid sequences other than any SEMA5B peptides. (e.g. random amino acid sequence). In a preferred embodiment, the immunological response of the subject is evaluated in a sequence specific manner, by comparison with an immunological response between each immunogen administered to the subject. In particular, even when a mixture of some kinds of SEMA5B peptides is administered to the subject, immunological response might vary depending on the peptides. In that case, by comparison of the immunological response between each peptide, peptides to which the subject shows higher response can be identified.

[0198] XII. Antibodies:

The present invention further provides antibodies that bind to peptides of the present invention. Preferred antibodies specifically bind to peptides of the present invention and will not bind (or will bind weakly) to other peptides. Alternatively, antibodies may bind to peptides of the present invention as well as the homologs thereof. Antibodies against peptides of the present invention can find use in cancer diagnostic and prognostic assays, as well as imaging methodologies. Similarly, such antibodies can find use in the treatment, diagnosis, and/or prognosis of other cancers, to the extent SEMA5B is also expressed or over-expressed in a cancer patient. Moreover, intracellularly expressed antibodies (e.g., single chain antibodies) may therapeutically find use in treating cancers in which the expression of SEMA5B is involved, example of which include, but are not limited to, esophageal cancer, NSCLC, RCC and SCLC.

[0199] The present invention also provides various immunological assays for the detection and/or quantification of the SEMA5B protein (SEQ ID NO: 75, 78 or 80) or fragments thereof, including polypeptides consisting of amino acid sequences selected from the group consisting of SEQ ID NOs: 2 to 69, preferably including polypeptides consisting of amino acid sequences selected from the group consisting of SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54. Such assays may include one or more anti-SEMA5B antibodies capable of recognizing and binding a SEMA5B protein or fragments thereof, as appropriate. In the context of the present invention, anti-SEMA5B antibodies binding to SEMA5B polypeptide preferably recognize polypeptide consisting of amino acid sequences selected from the group consisting of SEQ ID NOs: 2 to 69, in particular SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54, preferably to the exclusion of other peptides. The binding specificity of antibody can be confirmed by

means of an inhibition test. That is, when the binding between an antibody to be analyzed and full-length of SEMA5B polypeptide is inhibited under presence of any fragment polypeptides consisting of amino acid sequence of SEQ ID NOs: 2 to 69, it is deemed to specifically bind the fragment. In the context of the present invention, such immunological assays are performed within various immunological assay formats well known in the art, including but not limited to, various types of radioimmunoassays, immuno-chromatograph technique, enzyme-linked immunosorbent assays (ELISA), enzyme-linked immunofluorescent assays (ELIFA), and the like.

[0200] Related immunological but non-antibody assays of the invention may also include T cell immunogenicity assays (inhibitory or stimulatory) as well as MHC binding assays. In addition, the present invention contemplates immunological imaging methods capable of detecting cancers expressing SEMA5B, example of which include, but are not limited to, radioscintigraphic imaging methods using labeled antibodies of the present invention. Such assays find clinical use in the detection, monitoring, and prognosis of SEMA5B expressing cancers, examples of which include, but are not limited to, esophageal cancer, NSCLC, RCC and SCLC.

[0201] The present invention also provides antibodies that bind to the peptides of the present invention. An antibody of the present invention can be used in any form, for example as a monoclonal or polyclonal antibody, and may further include antiserum obtained by immunizing an animal such as a rabbit with the peptide of the present invention, all classes of polyclonal and monoclonal antibodies, human antibodies and humanized antibodies produced by genetic recombination.

[0202] A peptide of the present invention used as an antigen to obtain an antibody may be derived from any animal species, but is preferably derived from a mammal such as a human, mouse, or rat, more preferably from a human. A human-derived peptide may be obtained from the nucleotide or amino acid sequences disclosed herein.

[0203] According to the present invention, complete and partial peptides of the present invention may serve as immunization antigens. Examples of suitable partial peptides include, for example, the amino (N)-terminal or carboxy (C)-terminal fragment of a peptide of the present invention.

[0204] Herein, an antibody is defined as a protein that reacts with either the full length or a fragment of a SEMA5B peptide. In a preferred embodiment, an antibody of the present invention can recognize fragment peptides of SEMA5B having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 to 69, more preferably an amino acid sequence of SEQ ID NOs: 2, 3, 4, 8, 9, 10, 13, 20, 40, 41, 47 and 54. Methods for synthesizing oligopeptide are well known in the arts. After the synthesis, peptides may be optionally purified prior to use as immunogen. In the context of the present invention, the oligopeptide (e.g., 9- or 10mer) may be conjugated or linked

with carriers to enhance the immunogenicity. Keyhole-limpet hemocyanin (KLH) is well known as the carrier. Method for conjugating KLH and peptide are also well known in the arts.

[0205] Alternatively, a gene encoding a peptide of the present invention or fragment thereof may be inserted into a known expression vector, which is then used to transform a host cell as described herein. The desired peptide or fragment thereof may be recovered from the outside or inside of host cells by any standard method, and may subsequently be used as an antigen. Alternatively, whole cells expressing the peptide or their lysates or a chemically synthesized peptide may be used as the antigen.

[0206] Any mammalian animal may be immunized with the antigen, though preferably the compatibility with parental cells used for cell fusion is taken into account. In general, animals of Rodentia, Lagomorpha or Primates may be used. Animals of the family Rodentia include, for example, mouse, rat and hamster. Animals of the family Lagomorpha include, for example, rabbit. Animals of the Primate family include, for example, a monkey of Catarrhini (old world monkey) such as Macaca fascicularis, rhesus monkey, sacred baboon and chimpanzees.

[0207] Methods for immunizing animals with antigens are known in the art. Intraperitoneal injection or subcutaneous injection of antigens is a standard method for the immunization of mammals. More specifically, antigens may be diluted and suspended in an appropriate amount of phosphate buffered saline (PBS), physiological saline, etc. If desired, the antigen suspension may be mixed with an appropriate amount of a standard adjuvant, such as Freund's complete adjuvant, made into emulsion and then administered to mammalian animals. Preferably, it is followed by several administrations of antigen mixed with an appropriately amount of Freund's incomplete adjuvant every 4 to 21 days. An appropriate carrier may also be used for immunization. After immunization as described above, serum may be examined by a standard method for an increase in the amount of desired antibodies.

[0208] Polyclonal antibodies against the peptides of the present invention may be prepared by collecting blood from the immunized mammal examined for the increase of desired antibodies in the serum, and by separating serum from the blood by any conventional method. Polyclonal antibodies may include serum containing the polyclonal antibodies, as well as the fraction containing the polyclonal antibodies may be isolated from the serum. Immunoglobulin G or M can be prepared from a fraction which recognizes only a peptide of the present invention using, for example, an affinity column coupled with a peptide of the present invention, and further purifying this fraction using protein A or protein G column.

[0209] To prepare monoclonal antibodies for use in the context of the present invention, immune cells are collected from the mammal immunized with the antigen and checked

for the increased level of desired antibodies in the serum as described above, and are subjected to cell fusion. The immune cells used for cell fusion may preferably be obtained from spleen. Other preferred parental cells to be fused with the above immunocyte include, for example, myeloma cells of mammalians, and more preferably myeloma cells having an acquired property for the selection of fused cells by drugs.

[0210] The above immunocyte and myeloma cells can be fused according to known methods, for example, the method of Milstein et al. (Galfre and Milstein, Methods Enzymol 73: 3-46 (1981)).

[0211] Resulting hybridomas obtained by cell fusion may be selected by cultivating them in a standard selection medium, such as HAT medium (hypoxanthine, aminopterin and thymidine containing medium). The cell culture is typically continued in the HAT medium for several days to several weeks, the time being sufficient to allow all the other cells, with the exception of the desired hybridoma (non-fused cells), to die. Then, the standard limiting dilution may be performed to screen and clone a hybridoma cell producing the desired antibody.

[0212] In addition to the above method, wherein a non-human animal is immunized with an antigen for preparing hybridoma, human lymphocytes such as those infected by EB virus may be immunized with a peptide, peptide expressing cells or their lysates in vitro. Then, the immunized lymphocytes may be fused with human-derived myeloma cells that are capable of indefinitely dividing, such as U266, to yield a hybridoma producing a desired human antibody that is able to bind to the peptide can be obtained (Japanese Patent Application JPS 63-17688).

[0213] The obtained hybridomas may then be subsequently transplanted into the abdominal cavity of a mouse and the ascites extracted. The obtained monoclonal antibodies can be purified by, for example, ammonium sulfate precipitation, a protein A or protein G column, DEAE ion exchange chromatography or an affinity column to which a peptide of the present invention is coupled. An antibody of the present invention can be used not only for purification and detection of a peptide of the present invention, but also as a candidate for agonists and antagonists of a peptide of the present invention.

[0214] Alternatively, an immune cell, such as an immunized lymphocyte, producing antibodies may be immortalized by an oncogene and used for preparing monoclonal antibodies.

Monoclonal antibodies thus obtained can be also recombinantly prepared using genetic engineering techniques (see, for example, Borrebaeck and Larrick, Therapeutic Monoclonal Antibodies, published in the United Kingdom by MacMillan Publishers LTD (1990)). For example, a DNA encoding an antibody may be cloned from an immune cell, such as a hybridoma or an immunized lymphocyte producing the antibody, inserted into an appropriate vector, and introduced into host cells to prepare a

recombinant antibody. The present invention also provides for recombinant antibodies prepared as described above.

[0215] An antibody of the present invention may be a fragment of an antibody or modified antibody, so long as it binds to one or more of the peptides of the invention. For instance, the antibody fragment may be Fab, $F(ab')_2$, Fv or single chain Fv (scFv), in which Fv fragments from H and L chains are ligated by an appropriate linker (Huston et al., Proc Natl Acad Sci USA 85: 5879-83 (1988)). More specifically, an antibody fragment may be generated by treating an antibody with an enzyme, such as papain or pepsin. Alternatively, a gene encoding the antibody fragment may be constructed, inserted into an expression vector and expressed in an appropriate host cell (see, for example, Co et al., J Immunol 152: 2968-76 (1994); Better and Horwitz, Methods Enzymol 178: 476-96 (1989); Pluckthun and Skerra, Methods Enzymol 178: 497-515 (1989); Lamoyi, Methods Enzymol 121: 652-63 (1986); Rousseaux et al., Methods Enzymol 121: 663-9 (1986); Bird and Walker, Trends Biotechnol 9: 132-7 (1991)).

[0216] An antibody may be modified by conjugation with a variety of molecules, such as polyethylene glycol (PEG). The present invention provides for such modified antibodies. The modified antibody can be obtained by chemically modifying an antibody. These modification methods are conventional in the field.

[0217] Alternatively, an antibody of the present invention may be obtained as a chimeric antibody, between a variable region derived from nonhuman antibody and the constant region derived from human antibody, or as a humanized antibody, including the complementarity determining region (CDR) derived from nonhuman antibody, the frame work region (FR) and the constant region derived from human antibody. Such antibodies can be prepared according to known technology. Humanization can be performed by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody (see, e.g., Verhoeyen et al., Science 239:1534-1536 (1988)). Accordingly, such humanized antibodies are chimeric antibodies, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species.

[0218] Fully human antibodies including human variable regions in addition to human framework and constant regions can also be used. Such antibodies can be produced using various techniques known in the art. For example, in vitro methods involve use of recombinant libraries of human antibody fragments displayed on bacteriophage (e.g., Hoogenboom & Winter, J. Mol. Biol. 227:381 (1991). Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. This approach is described, e.g., in U.S. Patent Nos. 6,150,584, 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016.

[0219] Antibodies obtained as above may be purified to homogeneity. For example, the separation and purification of the antibody can be performed according to the separation and purification methods used for general proteins. For example, the antibody may be separated and isolated by the appropriately selected and combined use of column chromatographies, such as affinity chromatography, filter, ultrafiltration, salting-out, dialysis, SDS polyacrylamide gel electrophoresis and isoelectric focusing (Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988)), but are not limited thereto. A protein A column and protein G column can be used as the affinity column. Exemplary protein A columns to be used include, for example, Hyper D, POROS and Sepharose F.F. (Pharmacia).

[0220] Examples of suitable chromatography techniques, with the exception of affinity chromatography include, for example, ion-exchange chromatography, hydrophobic chromatography, gel filtration, reverse phase chromatography, adsorption chromatography and the like (Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Ed Daniel R. Marshak et al., Cold Spring Harbor Laboratory Press (1996)). The chromatographic procedures can be carried out by liquid-phase chromatography, such as HPLC and FPLC.

[0221] For example, measurement of absorbance, enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), radioimmunoassay (RIA) and/or immunofluorescence may be used to measure the antigen binding activity of the antibody of the present invention. In ELISA, the antibody of the present invention is immobilized on a plate, a peptide of the present invention is applied to the plate, and then a sample containing a desired antibody, such as culture supernatant of antibody producing cells or purified antibodies, is applied. Then, a secondary antibody that recognizes the primary antibody and is labeled with an enzyme, such as alkaline phosphatase, is applied, and the plate is incubated. Next, after washing, an enzyme substrate, such as p-nitrophenyl phosphate, is added to the plate, and the absorbance is measured to evaluate the antigen binding activity of the sample. A fragment of the peptide, such as a C-terminal or N-terminal fragment, may be used as the antigen to evaluate the binding activity of the antibody. BIAcore (Pharmacia) may be used to evaluate the activity of the antibody according to the present invention.

[0222] The above methods allow for the detection or measurement of a peptide of the present invention, by exposing an antibody of the invention to a sample presumed to contain a peptide of the present invention, and detecting or measuring the immune complex formed by the antibody and the peptide.

Because the method of detection or measurement of the peptide according to the invention can specifically detect or measure a peptide, the method can find use in a variety of experiments in which the peptide is used.

[0223] XIII. Vectors and Host Cells:

The present invention also provides for vectors and host cells into which a polynucleotide encoding a peptide of the present invention is introduced. A vector of the present invention finds utility as a carrier of nucleotides, especially a DNA, of the present invention in host cell, to express a peptide of the present invention, or to administer a polynucleotide of the present invention for gene therapy.

[0224] When E. coli is selected as the host cell and the vector is amplified and produced in a large amount in E. coli (e.g., JM109, DH5 alpha, HB101 or XL1Blue), the vector should have an "ori" to suitable for amplification in E. coli and a marker gene suited for selecting transformed E. coli (e.g., a drug-resistance gene selected by a drug such as ampicillin, tetracycline, kanamycin, chloramphenicol or the like). For example, M13-series vectors, pUC-series vectors, pBR322, pBluescript, pCR-Script, etc., can be used. In addition, pGEM-T, pDIRECT and pT7 can also be used for subcloning and extracting cDNA as well as the vectors described above. When a vector is used to produce the protein of the present invention, an expression vector can find use. For example, an expression vector to be expressed in E. coli should have the above characteristics to be amplified in E. coli. When E. coli, such as JM109, DH5 alpha, HB101 or XL1 Blue, are used as a host cell, the vector should have a promoter, for example, lacZ promoter (Ward et al., Nature 341: 544-6 (1989); FASEB J 6: 2422-7 (1992)), araB promoter (Better et al., Science 240: 1041-3 (1988)), T7 promoter or the like, that can efficiently express the desired gene in E. coli. In that respect, pGEX-5X-1 (Pharmacia), "QIAexpress system" (Qiagen), pEGFP and pET (in this case, the host is preferably BL21 which expresses T7 RNA polymerase), for example, can be used instead of the above vectors. Additionally, the vector may also contain a signal sequence for peptide secretion. An exemplary signal sequence that directs the peptide to be secreted to the periplasm of the E. coli is the pelB signal sequence (Lei et al., J Bacteriol 169: 4379 (1987)). Means for introducing of the vectors into the target host cells include, for example, the calcium chloride method, and the electroporation method.

[0225] In addition to E. coli, for example, expression vectors derived from mammals (for example, pcDNA3 (Invitrogen) and pEGF-BOS (Nucleic Acids Res 18(17): 5322 (1990)), pEF, pCDM8), expression vectors derived from insect cells (for example, "Bac-to-BAC baculovirus expression system" (GIBCO BRL), pBacPAK8), expression vectors derived from plants (e.g., pMH1, pMH2), expression vectors derived from animal viruses (e.g., pHSV, pMV, pAdexLcw), expression vectors derived from retroviruses (e.g., pZIpneo), expression vector derived from yeast (e.g., "Pichia Expression Kit" (Invitrogen), pNV11, SP-Q01) and expression vectors derived from Bacillus subtilis (e.g., pPL608, pKTH50) can be used for producing the polypeptide of the present invention.

[0226] In order to express the vector in animal cells, such as CHO, COS or NIH3T3 cells, the vector should have a promoter necessary for expression in such cells, for example, the SV40 promoter (Mulligan et al., Nature 277: 108 (1979)), the MMLV-LTR promoter, the EF1 alpha promoter (Mizushima et al., Nucleic Acids Res 18: 5322 (1990)), the CMV promoter and the like, and preferably a marker gene for selecting transformants (for example, a drug resistance gene selected by a drug (e.g., neomycin, G418)). Examples of known vectors with these characteristics include, for example, pMAM, pDR2, pBK-RSV, pBK-CMV, pOPRSV and pOP13.

[0227] Hereinafter, the present invention is described in more detail with reference to the Examples. However, while the following materials, methods and examples may serve to assist one of ordinary skill in making and using certain embodiments of the present invention, there are only intended to illustrate aspects of the present invention and thus in no way to limit the scope of the present invention. As one of ordinary skill in the art will readily recognize, methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention.

Examples

[0228] Experimental 1

Materials and Methods

Cell lines

TISI, HLA-A*2402-positive B-lymphoblastoid cell line, was purchased from IHWG Cell and Gene Bank (Seattle, WA). COS7, African green monkey kidney cell line, was purchased from ATCC.

[0229] Candidate selection of peptides derived from SEMA5B

9-mer and 10-mer peptides derived from SEMA5B that bind to HLA-A*2402 molecule were predicted using binding prediction software "BIMAS" (http://www-bimas.cit.nih.gov/molbio/hla_bind) (Parker et al.(J Immunol 1994, 152(1): 163-75), Kuzushima et al.(Blood 2001, 98(6): 1872-81)) and "NetMHC 3.2" (http://www.cbs.dtu.dk/services/NetMHC/) (Buus et al. (Tissue Antigens., 62:378-84, 2003), Nielsen et al. (Protein Sci., 12:1007-17, 2003, Bioinformatics, 20(9):1388-97, 2004)). These peptides were synthesized by Biosynthesis (Lewisville, Texas) 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 at 20 mg/ml and stored at -80 degrees C.

[0230] In vitro CTL Induction

Monocyte-derived dendritic cells (DCs) were used as antigen-presenting cells 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 isolated from a normal volunteer (HLA-A*2402 positive) by Ficoll-Paque plus (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 IU/ml of granulocyte-macrophage colony-stimulating factor (R&D System) and 1000 IU/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 micro g/ml of each of the synthesized peptides in the presence of 3 micro g/ml of beta 2-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 X ray-irradiated (20 Gy) 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 TISI 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).

[0231] 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 Mitomycin C, 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).

[0232] Establishment of CTL clones

The dilutions were made to have 0.3, 1, and 3 CTLs/well in 96 round-bottomed micro titer plate (Nalge Nunc International). CTLs were cultured with 1 x 10^4 cells/ well of 2 kinds of human B-lymphoblastoid cell lines, 30ng/ml of anti-CD3 antibody, and 125 IU/ml of IL-2 in a total of 150 micro l/well of AIM-V Medium containing 5%AS. 50 micro l /well of IL-2 were added to the medium 10 days later so to reach a final concentration of 125 IU/ml IL-2. CTL activity was tested on the 14th day, and CTL clones were expanded using the same method as described above (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).

[0233] Specific CTL activity

To examine specific CTL activity, IFN-gamma ELISPOT assays and IFN-gamma ELISA were performed. Peptide-pulsed TISI (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 were performed under manufacture procedure.

[0234] Establishment of the cells forcibly expressing either or both of the target gene and HLA-A24

The cDNA encoding an open reading frame of target genes or HLA-A*2402 was amplified by PCR. The PCR-amplified product was cloned into expression vector. The plasmids were transfected into COS7, which is the target genes and HLA-A*2402-null cell line, using lipofectamine 2000 (Invitrogen) according to the manufacturer's recommended procedures. After 2 days from transfection, the transfected cells were harvested with versene (Invitrogen) and used as the stimulator cells (5 x 10^4 cells/ well) for CTL activity assay.

[0235] Results

Enhanced SEMA5B expression in cancers

The wide gene expression profile data obtained from various cancers using cDNA-microarray revealed that SEMA5B (GenBank Accession No. NM_001031702; SEQ ID No: 74) expression was specifically elevated in cancer tissues as compared with corresponding normal tissue. SEMA5B expression was validly elevated in 1 out of 2 Esophageal Cancers, 1 out of 1 NSCLC, 14 out of 17 RCC and 4 out of 4 SCLC (Table 1).

[0236]

[Table 1]

Ratio of cases observed up-regulation of SEMA5B in cancerous tissue as compared with normal corresponding tissue.

Cancer/Tumor	Ratio
Esophageal Cancer	1/2
NSCLC	1/1
RCC	14/17
SCLC	4/4

[0237] Prediction of HLA-A24 binding peptides derived from SEMA5B

Table 2a and 2b show the HLA-A24 binding of 9mer and 10mer peptides of SEMA5B in the order of high binding affinity. A total of 69 peptides having potential HLA-A24 binding ability were selected and examined to determine the epitope peptides.

[0238]

[Table 2a]

HLA-A24 binding 9mer peptides derived from SEMA5B

Start position	Amino acid sequence	Kd (nM)	SEQ ID NO
247	LYAATVIDF	99	1
512	CYLEELHVL	244	2
1010	PYSEIPVIL	404	3
196	VFMCGTNAF	429	4
355	YYNELQSAF	502	5
139	IVGARNYLF	554	6
412	AWLPIANPI	583	7
723	IFWASWGSW	829	8
280	KWLNEPNFV	922	9
293	IGLFAYFFL	1370	10
374	VFTTNVNSI	1401	11
1093	YTPMEFKTL	1488	12
470	RFSHLVVDL	1570	13
371	IYGVFTTNV	1865	14
1126	TYYPSPLNK	2037	15
29	GWTVGGWLL	2062	16
533	ILHSARALF	3110	17
110	DLQPWVSNF	3118	18
590	NMSLWTQNI	3324	19
558	AYRSQGACL	3481	20
667	WTPWSSWAL	3501	21
1055	VYLSCQHCQ	3692	22
1122	VYTTTYYPS	3705	23
1099	KTLNKNNLI	3716	24
273	RTAQYNSKW	3825	25
291	YDIGLFAYF	3843	26
24	QQLRCGWTV	4294	27
525	REPLRSLRI	4296	28
1114	FYPLQQTNV	4449	29
144	NYLFRLSLA	4680	30
331	LLEDTWTTF	4761	31
490	YIGTESGTI	4985	32

Start position	Amino acid sequence	Binding score	SEQ ID NO
362	AFHLPEQDL	24	33
404	RYQENPRAA	18	34
440	RSLQDAQRL	14.4	35
1092	KYTPMEFKT	13.2	36
532	RILHSARAL	12	37
264	RSLGSGPPL	12	38
184	NYVRVLIVA	10.5	39

Start position indicates the number of amino acid residue from the N-terminus of SEMA5B.

Binding score and dissociation constant [Kd (nM)] are derived from "BIMAS" and "NetMHC3.2".

[0239] [Table 2b]

HLA-A24 binding 10mer peptides derived from SEMA5B

Start position	Amino acid sequence	Kd (nM)	SEQ ID NO
354	FYYNELQSAF	121	40
290	AYDIGLFAYF	136	41
404	RYQENPRAAW	294	42
1114	FYPLQQTNVY	329	43
666	AWTPWSSWAL	1299	44
291	YDIGLFAYFF	2015	45
138	LIVGARNYLF	2129	46
1044	CFLGSGLLTL	2237	47
119	TYPGARDFSQ	2264	48
335	TWTTFMKARL	2436	49
117	NFTYPGARDF	2683	50
532	RILHSARALF	2696	51
297	AYFFLRENAV	2699	52
330	FLLEDTWTTF	2919	53
489	LYIGTESGTI	3355	54
246	ELYAATVIDF	4738	55
440	RSLQDAQRLF	4857	56

Start position	Amino acid sequence	Binding score	SEQ ID NO
1092	KYTPMEFKTL	576	57
797	RFRFTCRAPL	40	58
286	NFVAAYDIGL	30	59
540	LFVGLRDGVL	30	60
1097	EFKTLNKNNL	24	61
500	KALSTASRSL	12	62
803	RAPLADPHGL	12	63
27	RCGWTVGGWL	11.2	64
898	EYQDCNPQAC	10.8	65
52	RTAEGPIMVL	9.6	66
747	RACENGNSCL	9.6	67
1127	YYPSPLNKHS	9	68
144	NYLFRLSLAN	9	69

Start position indicates the number of amino acid residue from the N-terminus of SEMA5B.

Binding score and dissociation constant [Kd (nM)] are derived from "BIMAS" and "NetMHC3.2".

[0240] CTL induction with the predicted peptides from SEMA5B restricted with HLA-A*2402

CTLs for those peptides derived from SEMA5B were generated according to the protocols as described in "Materials and Methods". Peptide specific CTL activity was detected by IFN-gamma ELISPOT assay (Figure 1a-l). The well number #7 with SEMA5B-A24-9-512 (SEQ ID NO: 2) (a), #3 with SEMA5B-A24-9-1010 (SEQ ID NO: 3) (b), #3 with SEMA5B-A24-9-196 (SEQ ID NO: 4) (c), #4 with SEMA5B-A24-9-723 (SEQ ID NO: 8) (d), #5 with SEMA5B-A24-9-280 (SEQ ID NO: 9) (e), #3 with SEMA5B-A24-9-293 (SEQ ID NO: 10) (f), #6 with SEMA5B-A24-9-470 (SEQ ID NO: 13) (g), #3 with SEMA5B-A24-9-558 (SEQ ID NO: 20) (h), #4 with SEMA5B-A24-10-354 (SEQ ID NO: 40) (i), #6 with SEMA5B-A24-10-290 (SEQ ID NO: 41) (j), #5 with SEMA5B-A24-10-1044 (SEQ ID NO: 47) (k) and #4 with SEMA5B-A24-10-489 (SEQ ID NO: 54) (l) demonstrated potent IFN-gamma production as compared to the control wells. On the other hand, no specific CTL activity was detected by stimulation with other peptides shown in Table 2a and b, despite those peptides had possible binding activity with HLA-A*2402. As is typical of negative data, no specific IFN-gamma production was observed from the CTL stimulated with SEMA5B-A24-9-247 (SEQ ID NO: 1) (m). Taken together, these results suggest that the 12 selected peptides derived from SEMA5B could induce potent CTLs.

[0241] Establishment of CTL line and clone against SEMA5B derived peptide

The cells that showed peptide specific CTL activity detected by IFN-gamma ELISPOT assay in the well number #7 with SEMA5B-A24-9-512 (SEQ ID NO: 2), #3 with SEMA5B-A24-9-1010 (SEQ ID NO: 3), #3 with SEMA5B-A24-9-293 (SEQ ID NO: 10) and #6 with SEMA5B-A24-10-290 (SEQ ID NO: 41) were expanded and established the CTL lines. CTL activity of these CTL lines was measured by IFN-gamma ELISA (Figure 2). CTL lines demonstrated potent IFN-gamma production against the target cells pulsed with the SEMA5B-A24-9-512 (SEQ ID NO: 2) (a), SEMA5B-A24-9-1010 (SEQ ID NO: 3) (b), SEMA5B-A24-9-293 (SEQ ID NO: 10) (c) and SEMA5B-A24-10-290 (SEQ ID NO: 41) (d) peptide as compared to target cells without peptide pulse. Furthermore, the CTL clones were established by limiting dilution from the CTL lines as described in "Materials and Methods", and IFN-gamma production from the CTL clones against target cells pulsed peptide was measured by IFN-gamma ELISA. Potent IFN-gamma production was observed from the CTL clones stimulated with SEMA5B-A24-9-512 (SEQ ID NO: 2) (a), SEMA5B-A24-9-1010 (SEQ ID NO: 3) (b) and SEMA5B-A24-10-290 (SEQ ID NO: 41) (c) (Figure 3).

[0242] Specific CTL activity against target cells expressing SEMA5B and HLA-A*2402

The established CTL line raised against SEMA5B-A24-10-290 (SEQ ID NO: 41) peptide was examined for the ability to recognize target cells that express SEMA5B and HLA-A*2402 molecule. COS7 cells transfected with both the full SEMA5B and HLA-A*2402 gene (a specific model for the target cells that express SEMA5B and HLA-A*2402 gene) were prepared as a stimulator cells, and COS7 cells transfected with either full length of SEMA5B or HLA-A*2402 were used as the controls. In Figure 4, the CTL line stimulated with SEMA5B-A24-10-290 (SEQ ID NO: 41) showed potent CTL activity against COS7 cells expressing both SEMA5B and HLA-A* 2402. On the other hand, no significant specific CTL activity was detected against the controls. Thus, this data clearly demonstrates that SEMA5B-A24-10-290 (SEQ ID NO: 41) peptide was endogenously processed and expressed on the target cells with HLA-A*2402 molecule and was recognized by the CTLs. These results indicated that this peptide derived from SEMA5B may be available to apply the cancer vaccines for patients with SEMA5B expressing tumors.

[0243] Homology analysis of antigen peptides

The CTLs stimulated with SEMA5B-A24-9-512 (SEQ ID NO: 2), SEMA5B-A24-9-1010 (SEQ ID NO: 3), SEMA5B-A24-9-196 (SEQ ID NO: 4), SEMA5B-A24-9-723 (SEQ ID NO: 8), SEMA5B-A24-9-280 (SEQ ID NO: 9), SEMA5B-A24-9-293 (SEQ ID NO: 10), SEMA5B-A24-9-470 (SEQ ID NO: 13), SEMA5B-A24-9-558 (SEQ ID NO: 20), SEMA5B-A24-10-354 (SEQ ID NO: 40),

SEMA5B-A24-10-290 (SEQ ID NO: 41), SEMA5B-A24-10-1044 (SEQ ID NO: 47) and SEMA5B-A24-10-489 (SEQ ID NO: 54) showed significant and specific CTL activity. These results may be due to the fact that the sequence of SEMA5B-A24-9-512 (SEQ ID NO: 2), SEMA5B-A24-9-1010 (SEQ ID NO: 3), SEMA5B-A24-9-196 (SEQ ID NO: 4), SEMA5B-A24-9-723 (SEQ ID NO: 8), SEMA5B-A24-9-280 (SEQ ID NO: 9), SEMA5B-A24-9-293 (SEQ ID NO: 10), SEMA5B-A24-9-470 (SEQ ID NO: 13), SEMA5B-A24-9-558 (SEQ ID NO: 20), SEMA5B-A24-10-354 (SEQ ID NO: 40), SEMA5B-A24-10-290 (SEQ ID NO: 41), SEMA5B-A24-10-1044 (SEQ ID NO: 47) and SEMA5B-A24-10-489 (SEQ ID NO: 54) are homologous to peptide derived from other molecules that are known to sensitize the human immune system. To exclude this possibility, homology analyses were performed for these peptide sequences using as queries the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi) which revealed no sequence with significant homology. The results of homology analyses indicate that the sequence of SEMA5B-A24-9-512 (SEQ ID NO: 2), SEMA5B-A24-9-1010 (SEQ ID NO: 3), SEMA5B-A24-9-196 (SEQ ID NO: 4), SEMA5B-A24-9-723 (SEQ ID NO: 8), SEMA5B-A24-9-280 (SEQ ID NO: 9), SEMA5B-A24-9-293 (SEQ ID NO: 10), SEMA5B-A24-9-470 (SEQ ID NO: 13), SEMA5B-A24-9-558 (SEQ ID NO: 20), SEMA5B-A24-10-354 (SEQ ID NO: 40), SEMA5B-A24-10-290 (SEQ ID NO: 41), SEMA5B-A24-10-1044 (SEQ ID NO: 47) and SEMA5B-A24-10-489 (SEQ ID NO: 54) are unique and thus, there is little possibility, to our best knowledge, that this molecules raise unintended immunologic response to some unrelated molecule.

[0244] In conclusion, the novel HLA-A*2402 epitope peptides derived from SEMA5B identified herein may find utility in the field of cancer immunotherapy.

Industrial Applicability

[0245] The present invention provides new epitope peptides derived from SEMA5B that may induce potent and specific anti-tumor immune responses and have applicability to a wide variety of cancer types. Such peptides can find use as peptide vaccines against diseases associated with SEMA5B, e.g., cancer, more particularly, esophageal cancer, NSCLC, RCC and SCLC.

[0246] While the present 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 present invention and its preferred embodiments. Through routine experimentation, one skilled in the art will readily recognize that various changes and modifications can be made therein without departing from the spirit and scope of the present invention, the metes and bounds of which are defined by the appended claims.

Claims

[Claim 1] An isolated peptide of less than 15 amino acids in length having cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide comprises an amino acid sequence (a) or (b) below:
(a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 41, 2, 3, 4, 8, 9, 10, 13, 20, 40, 47 and 54;
(b) an amino acid sequence in which 1, 2, or several amino acid(s) are substituted, deleted, inserted and/or added in the amino acid sequence selected from the group consisting of SEQ ID NOs: 41, 2, 3, 4, 8, 9, 10, 13, 20, 40, 47 and 54.

[Claim 2] The peptide of claim 1, wherein the peptide has one or both of the following characteristics:
(a) the second amino acid from the N-terminus is phenylalanine, tyrosine, methionine or tryptophan; and
(b) the C-terminal amino acid is phenylalanine, leucine, isoleucine, tryptophan or methionine.

[Claim 3] The peptide of claim 1 or 2, wherein the peptide is a nonapeptide or a decapeptide.

[Claim 4] An isolated polynucleotide encoding the peptide of any one of claims 1 to 3.

[Claim 5] A composition for inducing a CTL, wherein the composition comprises at least one active ingredient selected from the group consisting of:
(a) the peptide of any one of claims 1 to 3;
(b) the polynucleotide of claim 4;
(c) an antigen-presenting cell (APC) that presents the peptide of any one of claims 1 to 3 on its surface; and
(d) an exosome that presents the peptide of any one of claims 1 to 3 on its surface.

[Claim 6] A pharmaceutical composition for the treatment and/or prophylaxis of cancer, and/or the prevention of a postoperative recurrence thereof, wherein the composition comprises at least one active ingredient selected from the group consisting of:
(a) the peptide of any one of claims 1 to 3;
(b) the polynucleotide of claim 4;
(c) an APC that presents the peptide of any one of claims 1 to 3 on its surface;
(d) an exosome that presents the peptide of any one of claims 1 to 3 on

its surface; and

(e) a CTL that can recognize a cell presenting the peptide of any one of claims 1 to 3.

[Claim 7] The pharmaceutical composition of claim 6, wherein the pharmaceutical composition is formulated for the administration to a subject whose HLA antigen is HLA-A24.

[Claim 8] A method for inducing an APC with CTL inducibility, wherein the method comprises a step selected from the group consisting of:

(a) contacting an APC with the peptide of any one of claims 1 to 3; and

(b) introducing a polynucleotide encoding the peptide of any one of claims 1 to 3 into an APC.

[Claim 9] A method for inducing a CTL, wherein the method comprises a step selected from the group consisting of:

(a) co-culturing a CD8 positive T cell with an APC that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 3;

(b) co-culturing a CD8 positive T cell with an exosome that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 3; and

(c) introducing into a CD8 positive T cell a polynucleotide encoding both of T cell receptor (TCR) subunits or polynucleotides encoding each of TCR subunits, wherein the TCR formed by said subunits can bind to a complex of the peptide of any one of claims 1 to 3 and an HLA antigen on a cell surface.

[Claim 10] An isolated APC that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 3.

[Claim 11] The APC of claim 10, which is induced by the method of claim 8.

[Claim 12] An isolated CTL that targets the peptide of any one of claims 1 to 3.

[Claim 13] The CTL of claim 12, which is induced by the method of claim 9.

[Claim 14] A method of inducing an immune response against cancer in a subject, wherein the method comprises the step of administering to the subject a composition comprising the peptide of any one of claims 1 to 3, or a polynucleotide encoding the peptide.

[Claim 15] An antibody or immunologically active fragment thereof against the peptide of any one of claims 1 to 3.

[Claim 16] A vector comprising a nucleotide sequence encoding the peptide of any one of claims 1 to 3.

[Claim 17] A host cell transformed or transfected with the vector of claim 16.

[Claim 18] A diagnostic kit comprising the peptide of any one of claims 1 to 3, the polynucleotide of claim 4 or the antibody or immunologically active fragment of claim 15.

[Claim 19] A method of screening for a peptide having an ability to induce a CTL that has specific cytotoxic activity against a cell that presents a fragment derived from SEMA5B, wherein the method comprises the steps of:

(i) providing a candidate sequence consisting of an amino acid sequence modified by substituting, deleting, inserting and/or adding one, two or several amino acid residues to an original amino acid sequence, wherein the original amino acid sequence is selected from the group consisting of SEQ ID NOs: 41, 2, 3, 4, 8, 9, 10, 13, 20, 40, 47 and 54;

(ii) selecting a candidate sequence that does not have substantial significant homology with the peptides derived from any known human gene products other than SEMA5B;

(iii) contacting a peptide consisting of the candidate sequence selected in step (ii) with an antigen presenting cell;

(iv) contacting the antigen presenting cell of step (iii) with a CD8 positive T cell; and

(v) identifying the peptide of which CTL inducibility is same to or higher than a peptide consisting of the original amino acid sequence.