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(54) Title: ELOVL7 EPITOPE PEPTIDES AND VACCINES CONTAINING THE SAME

(57) Abstract: Peptide vaccines against cancer are described herein. In particular, the present invention provides epitope peptides derived from ELOVL7 that elicit CTLs. Antigen-presenting cells and an isolated CTL that targets the peptides, as well as methods for inducing the antigen-presenting cell, or CTL are also provided. The present invention further provides pharmaceutical agents containing the ELOVL7 polypeptides or polynucleotides encoding thereof as active ingredients. Furthermore, the present invention provides methods for treating and/or prophylaxis of (i.e., preventing) cancers (tumors), and/or prevention of postoperative recurrence thereof, as well as methods for inducing CTLs, methods for inducing anti-tumor immunity, using the ELOVL7 polypeptides, polynucleotides encoding the polypeptides, or antigen-presenting cells presenting the polypeptides, or the pharmaceutical agents of the present invention.

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Description

Title of Invention: ELOVL7 EPITOPE PEPTIDES AND VACCINES CONTAINING THE SAME

Technical Field

[0001] The present application claims the benefit of U.S. Provisional Application No. 61/201,948, filed on December 16, 2008, the entire contents of which are incorporated by reference herein.

[0002] Technical Field

The present invention relates to the field of biological science, more specifically to the field of cancer therapy. In particular, the present invention relates to novel peptides that are extremely effective as cancer vaccines, and drugs for treating and preventing tumors.

Background Art

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

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

[0005] Elongation of very long chain fatty acids (ELOVL) family proteins belong to a highly conserved family of microsomal enzymes involved in the formation of very-long-chain fatty acids (NPL 14/Leonard et al., Prog Lipid Res. 2004 Jan;43(1):36-54). Of these, ELOVL1, 3 and 6 are suggested to be involved in the elongation of saturated and monounsaturated very-long-chain fatty acids. On the other hand, ELOVL2, 4 and 5 are elongases of polyunsaturated fatty acids (NPL 15/Jakobsson et al., Prog Lipid Res. 2006 May;45(3):237-49). ELOVL7, an ELOVL family protein consisting of 281 amino acids and having 47.6 % homology to ELOVL1, has been identified as a gene over expressed in prostate cancer (NPL 16/NCBI accession ID: AB181393, Tamura et al., 2005).

Citation List

Non Patent Literature

[0006] NPL 1: Boon T, Int J Cancer 1993 May 8, 54(2): 177-80
NPL 2: Boon T & van der Bruggen P, J Exp Med 1996 Mar 1, 183(3): 725-9
NPL 3: Harris CC, J Natl Cancer Inst 1996 Oct 16, 88(20): 1442-55
NPL 4: Butterfield LH et al., Cancer Res 1999 Jul 1, 59(13): 3134-42
NPL 5: Vissers JL et al., Cancer Res 1999 Nov 1, 59(21): 5554-9
NPL 6: van der Burg SH et al., J Immunol 1996 May 1, 156(9): 3308-14
NPL 7: Tanaka F et al., Cancer Res 1997 Oct 15, 57(20): 4465-8
NPL 8: Fujie T et al., Int J Cancer 1999 Jan 18, 80(2) 169-72
NPL 9: Kikuchi M et al., Int J Cancer 1999 May 5, 81(3): 459-66
NPL 10: Oiso M et al., Int J Cancer 1999 May 5, 81(3): 387-94
NPL 11: Belli F et al., J Clin Oncol 2002 Oct 15, 20(20): 4169-80
NPL 12: Coulie PG et al., Immunol Rev 2002 Oct, 188: 33-42
NPL 13: Rosenberg SA et al., Nat Med 2004 Sep, 10(9): 909-15
NPL 14: Leonard et al., Prog Lipid Res. 2004 Jan;43(1):36-54
NPL 15: Jakobsson et al., Prog Lipid Res. 2006 May;45(3):237-49
NPL 16: NCBI accession ID: AB181393, Tamura et al., 2005

Summary of Invention

[0007] The present invention is based in part on the discovery of suitable epitope peptides that may serve as targets of immunotherapy. Because TAAs are generally perceived by the immune system as "self" and therefore often have no innate immunogenicity, the discovery of appropriate targets is of extreme importance. As noted above, ELOVL7 (SEQ ID NO: 29 encoded by the gene of GenBank Accession No. AB181393, NM_001104558 or NM_024930 (for example, SEQ ID NO: 28)) has been identified as up-regulated in many types of cancers, including bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, non-small cell lung cancer

(NSCLC), prostate cancer, renal carcinoma and small cell lung cancer (SCLC). Thus, the present invention focuses on ELOVL7 as a candidate target for immunotherapy, more particularly on the identification of specific epitope peptides of the gene products of ELOVL7 that possess the ability to induce CTLs specific to ELOVL7. As discussed in detail below, peripheral blood mononuclear cells (PBMCs) obtained from a healthy donor were stimulated using HLA-A*2402 binding candidate peptides derived from ELOVL7. CTL lines were then established with specific cytotoxicity against the HLA-A24 positive target cells pulsed with each of candidate peptides. These results demonstrate that these peptides are HLA-A24 restricted epitope peptides that can induce potent and specific immune responses against cells expressing ELOVL7. Further, the results indicate that ELOVL7 is strongly immunogenic and that the epitopes thereof are effective targets for tumor immunotherapy.

[0008] Accordingly, it is an object of the present invention to provide isolated peptides that bind to HLA antigen, particularly those that consist of ELOVL7 (SEQ ID NO: 29) or an immunogenic fragment. These peptides are expected to have CTL inducibility and, thus, can be used to induce CTL ex vivo or to be administered to a subject for inducing immune responses against cancers such as bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, NSCLC, prostate cancer, renal carcinoma and SCLC. Preferred peptides are nonapeptides or decapeptides, and more preferably those having an amino acid sequence selected from among from among SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 14, 16, 18, 19, 20, 21, 22, 25, 26 and 27, were demonstrated to show strong CTL inducibility.

[0009] The peptides of the invention encompass those wherein one, two or more amino acids are substituted, inserted, deleted or added, so long as the resulting modified peptides retain the original CTL inducibility.

[0010] The present invention also provides isolated polynucleotides encoding any peptides of the present invention. These polynucleotides can be used to induce APCs with CTL inducibility or can be administered to a subject for inducing immune responses against cancers much like the present peptides.

[0011] When administered to a subject, the present peptides are preferably presented on the surface of APCs so as to induce CTLs targeting the respective peptides. Therefore, one aspect of the present invention relates to agents containing any peptides or polynucleotides of the present invention for inducing CTL. The present invention contemplates the use of such agents containing any peptides or polynucleotides in the treatment and/or prophylaxis of cancers, such as bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, NSCLC, prostate cancer, renal carcinoma and SCLC, and/or preventing postoperative recurrence thereof. Thus, the present invention also provides pharmaceutical agents for the treatment and/or pro-

phylaxis of cancers, and/or for preventing the postoperative recurrence thereof, such pharmaceutical agents including any peptides or polynucleotides of the present invention as an active ingredient. Examples of such agents or pharmaceutical agents of the present invention include APCs or exosomes that present any of the present peptides instead of or in addition to the present peptides or polynucleotides as active ingredients.

[0012] The peptides or polynucleotides of the present invention can induce APCs that present on their surface a complex of an HLA antigen and the present peptide, for example, by contacting APCs derived from a subject with the peptide or introducing the polynucleotide encoding the peptide of the present invention into APCs. Such APCs have high CTL inducibility against target peptides and are therefore useful for cancer immunotherapy. Accordingly, the present invention extends to methods for inducing APCs with CTL inducibility and the APCs obtained by the methods.

[0013] It is yet another object of the present invention to provide methods for inducing CTL, such methods including the step of co-culturing CD8-positive cells with APCs or exosomes that present on its surface a peptide of the present invention or the step introducing a gene composed of a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide binding to a present peptide. The CTLs obtained by such methods are useful in the treatment and/or prevention of many cancers, including, for example, bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, NSCLC, prostate cancer, renal carcinoma and SCLC. Therefore, the present invention encompasses the CTLs obtained by the present methods.

[0014] It is a further object of the present invention to provide methods for inducing immune response against cancers, such methods including the step of administering agents containing the ELOVL7 polypeptides, polynucleotides encoding ELOVL7 polypeptides, exosomes or the APCs presenting ELOVL7 polypeptides.

[0015] The present invention extends to the treatment and prevention of any of diseases relating to ELOVL7 over-expression, such as cancer, exemplary bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, NSCLC, prostate cancer, renal carcinoma and SCLC.

[0016] In addition to the above, other 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. However, it is to be understood that both the foregoing summary of the invention and the following detailed description are of exemplified embodiments, and not restrictive of the invention or other alternate embodiments of the invention. 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 advantages will be apparent from the above in conjunction with the accompanying examples, data, figures and all reasonable inferences to be drawn therefrom, alone or with consideration of the references incorporated herein.

Brief Description of Drawings

[0017] Various aspects and applications of the present invention will become apparent to the skilled artisan upon consideration of the brief description of the figures and the detailed description of the present invention and its preferred embodiments that follows.

[fig.1a-h]Figure 1 is composed of a series of photographs (a) - (q), depicting the results of an IFN-gamma ELISPOT assay on CTLs that were induced with peptides derived from ELOVL7. The CTLs in the following well numbers showed potent IFN-gamma production as compared with the control: well number #6 stimulated with ELOVL7-A24-9-120 (SEQ ID NO: 3) (a), with well number #5 stimulated with ELOVL7-A24-9-244 (SEQ ID NO: 4) (b), well number #1 stimulated with ELOVL7-A24-9-70 (SEQ ID NO: 5) (c), well number #7 stimulated with ELOVL7-A24-9-80 (SEQ ID NO: 6) (d), well number #5 stimulated with ELOVL7-A24-9-246 (SEQ ID NO: 7) (e), well number #1 stimulated with ELOVL7-A24-9-248 (SEQ ID NO: 8) (f), well number #5 stimulated with ELOVL7-A24-9-124 (SEQ ID NO: 9) (g), well number #2 stimulated with ELOVL7-A24-9-214 (SEQ ID NO: 14) (h). The cells in the wells denoted with a rectangular box were expanded to establish CTL lines. 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.

[fig.1i-q]Figure 1 is composed of a series of photographs (a) - (q), depicting the results of an IFN-gamma ELISPOT assay on CTLs that were induced with peptides derived from ELOVL7. The CTLs in the following well numbers showed potent IFN-gamma production as compared with the control: well numbers #1 and #6 stimulated with ELOVL7-A24-10-232 (SEQ ID NO: 16) (i), well number #2 stimulated with ELOVL7-A24-10-184 (SEQ ID NO: 18) (j), well number #2 stimulated with ELOVL7-A24-10-244 (SEQ ID NO: 19) (k), well number #1 stimulated with ELOVL7-A24-10-124 (SEQ ID NO: 20) (l), well number #2 stimulated with ELOVL7-A24-10-46 (SEQ ID NO: 21) (m), well numbers #3, #6 and #7 stimulated

with ELOVL7-A24-10-42 (SEQ ID NO: 22) (n), well number #5 with ELOVL7-A24-10-199 (SEQ ID NO: 25) (o), well number #5 stimulated with ELOVL7-A24-10-77 (SEQ ID NO: 26) (p) and well number #4 stimulated with ELOVL7-A24-10-70 (SEQ ID NO: 27) (q). The cells in the wells denoted with a rectangular box were expanded to establish CTL lines. 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.

[fig.2a-h]Figure 2 is composed of a series of line graphs, (a) to (l), depicting the results of an IFN-gamma ELISA assay demonstrating the IFN-gamma production of CTL lines stimulated with ELOVL7-A24-9-120 (SEQ ID NO: 3) (a), with ELOVL7-A24-9-244 (SEQ ID NO: 4) (b), with ELOVL7-A24-9-70 (SEQ ID NO: 5) (c), with ELOVL7-A24-9-80 (SEQ ID NO: 6) (d), with ELOVL7-A24-10-232 (SEQ ID NO: 16) (e), with ELOVL7-A24-10-184 (SEQ ID NO: 18) (f), with ELOVL7-A24-10-244 (SEQ ID NO: 19) (g), with ELOVL7-A24-10-46 (SEQ ID NO: 21) (h). 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.

[fig.2i-l]Figure 2 is composed of a series of line graphs, (a) to (l), depicting the results of an IFN-gamma ELISA assay demonstrating the IFN-gamma production of CTL lines stimulated with ELOVL7-A24-10-42 (SEQ ID NO: 22) (i), with ELOVL7-A24-10-199 (SEQ ID NO: 25) (j), with ELOVL7-A24-10-77 (SEQ ID NO: 26) (k) and with ELOVL7-A24-10-70 (SEQ ID NO: 27) (l). 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.

[fig.3]Figure 3 is composed of a series of line graphs, (a) to (h), depicting the IFN-gamma production of the CTL clones established by limiting dilution from the CTL lines stimulated with ELOVL7-A24-9-120 (SEQ ID NO: 3) (a), ELOVL7-A24-9-70 (SEQ ID NO: 5) (b), ELOVL7-A24-9-80 (SEQ ID NO: 6) (c), ELOVL7-A24-10-232 (SEQ ID NO: 16) (d), ELOVL7-A24-10-184 (SEQ ID NO: 18) (e), ELOVL7-A24-10-244 (SEQ ID NO: 19) (f), ELOVL7-A24-10-42 (SEQ ID NO: 22) (g) and ELOVL7-A24-10-77 (SEQ ID NO: 26) (h). The results demonstrate that the CTL clones established by stimulation with SEQ ID NO: 3 (a), SEQ ID NO: 5 (b), SEQ ID NO: 6 (c), SEQ ID NO: 16 (d), SEQ ID NO: 18 (e), SEQ ID NO: 19 (f), SEQ ID NO: 22 (g) and SEQ ID NO: 26 (h) showed potent IFN-gamma production

compared with the control. In the figure, "+" indicates the IFN-gamma production against target cells pulsed with SEQ ID NO: 3 (a), SEQ ID NO: 6 (b), SEQ ID NO: 7 (c), SEQ ID NO: 17 (d), SEQ ID NO: 19 (e), SEQ ID NO: 20 (f), SEQ ID NO: 23 (g) and SEQ ID NO: 26 (h) and "-" indicates the IFN-gamma production against target cells not pulsed with any peptides.

[fig.4]Figure 4 is composed of a series of line graphs, (a) to (c), depicting the specific CTL activity against the target cells that exogenously express ELOVL7 and HLA-A*2402. COS7 cells transfected with HLA-A*2402 or with the full length ELOVL7 gene were prepared as control. The CTL clones established with ELOVL7-A24-9-120 (SEQ ID NO: 3) (a), ELOVL7-A24-9-70 (SEQ ID NO: 5) (b) and ELOVL7-A24-9-80 (SEQ ID NO: 6) (c) showed specific CTL activity against COS7 cells transfected with both ELOVL7 and HLA-A*2402 (black lozenge). On the other hand, no significant specific CTL activity was detected against target cells expressing either HLA-A*2402 (triangle) or ELOVL7 (circle).

Description of Embodiments

[0018] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the present invention, the preferred methods, devices, and materials are now described. However, before the present materials and methods are described, it is to be understood that the present invention is not limited to the particular sizes, shapes, dimensions, materials, methodologies, protocols, etc. described herein, as these may vary in accordance with routine experimentation and optimization. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[0019] The disclosure of each publication, patent or patent application mentioned in this specification is specifically incorporated by reference herein in its entirety. However, nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0020] I. Definitions

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. However, in case of conflict, the present specification, including definitions, will control.

[0021] The words "a", "an", and "the" as used herein mean "at least one" unless otherwise specifically indicated.

[0022] The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to

refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is a modified residue, or a non-naturally occurring residue, such as an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

[0023] The term "amino acid" as used herein refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that similarly function to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those modified after translation in cells (e.g., hydroxyproline, gamma-carboxyglutamate, and O-phosphoserine). The phrase "amino acid analog" refers to compounds that have the same basic chemical structure (an alpha carbon bound to a hydrogen, a carboxy group, an amino group, and an R group) as a naturally occurring amino acid but have a modified R group or modified backbones (e.g., homoserine, norleucine, methionine, sulfoxide, methionine methyl sulfonium). The phrase "amino acid mimetic" refers to chemical compounds that have different structures but similar functions to general amino acids.

[0024] 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.

[0025] The terms "gene", "polynucleotides", "nucleotides" and "nucleic acids" are used interchangeably herein and, unless otherwise specifically indicated, referred to by their commonly accepted single-letter codes.

[0026] Unless otherwise defined, the term "cancer" refers to the cancers over-expressing the ELOVL7 gene, examples of which include bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, NSCLC, prostate cancer, renal carcinoma and SCLC.

[0027] 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.

[0028] Unless otherwise defined, the term "cytotoxic T lymphocyte", "cytotoxic T cell" and "CTL" are used interchangeably herein and, unless otherwise specifically indicated, refer to a sub-group of T lymphocytes that are capable of recognizing non-self cells (e.g., tumor cells, virus-infected cells) and inducing the death of such cells.

[0029] II. Peptides

To demonstrate that peptides derived from ELOVL7 function as an antigen recognized by CTLs, peptides derived from ELOVL7 (SEQ ID NO: 29) 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 ELOVL7 were identified based on their binding affinities to HLA-A24. After in vitro stimulation of T-cells by dendritic cells (DCs) loaded with these peptides, CTLs are successfully established using each of the following peptides;

ELOVL7-A24-9-120 (SEQ ID NO: 3),
ELOVL7-A24-9-244 (SEQ ID NO: 4),
ELOVL7-A24-9-70 (SEQ ID NO: 5),
ELOVL7-A24-9-80 (SEQ ID NO: 6),
ELOVL7-A24-9-246 (SEQ ID NO: 7),
ELOVL7-A24- 9-248 (SEQ ID NO: 8),
ELOVL7-A24- 9-124 (SEQ ID NO: 9),
ELOVL7-A24-9-214 (SEQ ID NO: 14),
ELOVL7-A24-10-232 (SEQ ID NO: 16),
ELOVL7-A24-10-184 (SEQ ID NO: 18),
ELOVL7-A24-10-244 (SEQ ID NO: 19),
ELOVL7-A24-10-124 (SEQ ID NO: 20),
ELOVL7-A24-10-46 (SEQ ID NO: 21),
ELOVL7-A24-10-42 (SEQ ID NO: 22),
ELOVL7-A24-10-199 (SEQ ID NO: 25),
ELOVL7-A24-10-77 (SEQ ID NO: 26), and
ELOVL7-A24-10-70 (SEQ ID NO: 27).

[0030] These established CTLs show potent specific CTL activity against target cells pulsed with respective peptides. These results herein demonstrate that ELOVL7 is an antigen recognized by CTL and that the peptides are epitope peptides of ELOVL7 restricted by HLA-A24.

[0031] Since the ELOVL7 gene is over expressed in cancer cells and tissues, including for example those of bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, NSCLC, prostate cancer, renal carcinoma and SCLC and not expressed in most normal organs, it represents a good target for immunotherapy. Thus, the present invention provides nonapeptides (peptides consisting of nine amino acid residues) and decapeptides (peptides consisting of ten amino acid residues) corresponding to CTL-recognized epitopes from ELOVL7. Particularly preferred examples of nonapeptides and decapeptides of the present invention include those peptides having an amino acid sequence selected among SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 14, 16, 18, 19, 20, 21, 22, 25, 26 and 27.

[0032] Generally, software programs presently available on the Internet, such as those described in Parker KC et al., J Immunol 1994 Jan 1, 152(1): 163-75, can be used to calculate the binding affinities between various peptides and HLA antigens in silico.

Binding affinity with HLA antigens can be measured as described, for example, in Parker KC et al., J Immunol 1994 Jan 1, 152(1): 163-75; and Kuzushima K et al., Blood 2001, 98(6): 1872-81. The methods for determining binding affinity is described, for example, in the Journal of Immunological Methods, 1995, 185: 181-190, and Protein Science, 2000, 9: 1838-1846. Therefore, one can select fragments derived from ELOVL7, which have high binding affinity with HLA antigens using such software programs. Thus, the present invention encompasses peptides consisting of any fragments derived from ELOVL7, that bind with HLA antigens identified using such known programs. Furthermore, such peptides can include the peptide consisting of the full length of ELOVL7.

[0033] The nonapeptides and decapeptides of the present invention can be flanked with additional amino acid residues, so long as the resulting peptide retains its CTL inducibility. The additional amino acid residues can 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 with binding affinity to HLA antigens, including peptides derived from ELOVL7. 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.

[0034] In general, the modification of one, two, or more amino acids in a peptide will not influence the function of the peptide, and in some cases will even enhance the desired function of the original protein. In fact, modified peptides (i.e., peptides composed of an amino acid sequence in which one, two or several amino acid residues have been modified (i.e., substituted, added or inserted) as compared to an original reference sequence) have been known to retain the biological activity of the original peptide (Mark et al., Proc Natl Acad Sci USA 1984, 81: 5662-6; Zoller and Smith, Nucleic Acids Res 1982, 10: 6487-500; Dalbadie-McFarland et al., Proc Natl Acad Sci USA 1982, 79: 6409-13). Thus, in one embodiment, the peptides of the present invention have both CTL inducibility and having an amino acid sequence selected from among SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 14, 16, 18, 19, 20, 21, 22, 25, 26 and 27, wherein one, two or even more amino acids are added, inserted, deleted, and/or substituted.

[0035] Those of skill in the art recognize that individual additions or substitutions to an amino acid sequence which alters a single amino acid or a small percentage of amino acids tend to result in the conservation of the properties of the original amino acid side-chain. As such, they are often referred to as "conservative substitutions" or "conservative modifications", wherein the alteration of a protein results in a modified protein with having a function analogous to the original protein. Conservative substitution tables providing functionally similar amino acids are well known in the art. Examples of amino acid side chain characteristics that are desirable to conserve

include, for example, hydrophobic amino acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), and side chains having the following functional groups or characteristics in common: an aliphatic side-chain (G, A, V, L, I, P); a hydroxyl group containing side-chain (S, T, Y); a sulfur atom containing side-chain (C, M); a carboxylic acid and amide containing side-chain (D, N, E, Q); a base containing side-chain (R, K, H); and an aromatic containing side-chain (H, F, Y, W). In addition, the following eight groups each contain amino acids that are accepted in the art as conservative substitutions for one another:

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

[0036] Such conservatively modified peptides are also considered to be peptides of the present invention. However, peptides of the present invention are not restricted thereto and can include non-conservative modifications, so long as the modified peptide retains the CTL inducibility of the original peptide. Furthermore, modified peptides should not exclude CTL inducible peptides of polymorphic variants, interspecies homologues, and alleles of ELOVL7.

[0037] To retain the requisite CTL inducibility one can modify (insert, add add/or substitute) a small number (for example, 1, 2 or several) or a small percentage of amino acids. Herein, the term "several" means 5 or fewer amino acids, for example, 4 or 3 or fewer. The percentage of amino acids to be modified is preferably 20% or less, more preferably, 15% of less, even more preferably 10% or less or 1 to 5%.

[0038] When used in the context of immunotherapy, peptides of the present invention should be presented on the surface of a cell or exosome, preferably as a complex with an HLA antigen. Therefore, it is preferable to select peptides that not only induce CTLs but also possess high binding affinity to the HLA antigen. To that end, the peptides can be modified by substitution, insertion, and/or addition of the amino acid residues to yield a modified peptide having improved binding affinity. In addition to peptides that are naturally displayed, since the regularity of the sequences of peptides displayed by binding to HLA antigens is already known (J Immunol 1994, 152: 3913; Immunogenetics 1995, 41: 178; J Immunol 1994, 155: 4307), modifications based on such regularity can be introduced into the immunogenic peptides of the invention. For example, it may be desirable to substitute the second amino acid from the N-terminus

with phenylalanine, tyrosine, methionine, or tryptophan, and 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 the amino acid sequences selected from among SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 14, 16, 18, 19, 20, 21, 22, 25, 26 and 27 wherein 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 wherein 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. Substitutions can be introduced not only at the terminal amino acids but also at the position of potential T cell receptor (TCR) recognition of peptides. Several studies have demonstrated that a peptide with amino acid substitutions can be equal to or better than 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) Feb 1;168(3):1338-47., S. O. Dionne et al. Cancer Immunol immunother. (2003) 52: 199-206 and S. O. Dionne et al. Cancer Immunology, Immunotherapy (2004) 53, 307-314).

[0039] The present invention also contemplates the addition of one, two or several amino acids to the N and/or C-terminus of the described peptides. Such modified peptides having high HLA antigen binding affinity and retained CTL inducibility are also included in the present invention.

[0040] However, when the peptide sequence is identical to a portion of the amino acid sequence of an endogenous or exogenous protein having a different function, side effects such as autoimmune disorders and/or allergic symptoms against specific substances may be induced. Therefore, it is preferable to first perform homology searches using available databases to avoid situations in which the sequence of the peptide matches the amino acid sequence of another protein. When it becomes clear from the homology searches that there exists not even a peptide with 1 or 2 amino acid differences as compared to the objective peptide, the objective peptide can be modified in order to increase its binding affinity with HLA antigens, and/or increase its CTL inducibility without any danger of such side effects.

[0041] Although peptides having high binding affinity to the HLA antigens as described above are expected to be highly effective, the candidate peptides, which are selected according to the presence of high binding affinity as an indicator, are further examined for the presence of CTL inducibility. Herein, the phrase "CTL inducibility" indicates the ability of the peptide to induce cytotoxic lymphocytes (CTLs) when presented on antigen-presenting cells (APCs). Further, "CTL inducibility" includes the ability of the peptide to induce CTL activation, CTL proliferation, promote CTL lysis of target cells, and to increase CTL IFN-gamma production.

[0042] 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 with the peptides, mixing with CD8-positive cells, and then measuring the IFN-gamma produced and released by CTL against the target cells. As the reaction system, transgenic animals that have been produced to express a human HLA antigen (for example, those described in BenMohamed L, Krishnan R, Longmate J, Auge C, Low L, Primus J, Diamond DJ, Hum Immunol 2000 Aug, 61(8): 764-79, Related Articles, Books, Linkout Induction of CTL response by a minimal epitope vaccine in HLA A*0201/DR1 transgenic mice: dependence on HLA class II restricted T(H) response) can be used. For example, the target cells can be radiolabeled with ^{51}Cr and such, and cytotoxic activity can be calculated from radioactivity released from the target cells. Alternatively, CTL inducibility can be assessed can be examined by measuring IFN-gamma produced and released by CTL in the presence of APCs that carry immobilized peptides, and visualizing the inhibition zone on the media using anti-IFN-gamma monoclonal antibodies.

[0043] As a result of examining the CTL inducibility of the peptides as described above, it was discovered that nonapeptides or decapeptides selected from among the amino acid sequences indicated by SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 14, 16, 18, 19, 20, 21, 22, 25, 26 and 27 showed particularly high CTL inducibility as well as high binding affinity to an HLA antigen. Thus, these peptides are exemplified preferred embodiments of the present invention.

[0044] Furthermore, the result of homology analysis showed that those peptides do not have significant homology with peptides derived from any other known human gene products. Accordingly, the possibility of unknown or undesired immune responses arising when used for immunotherapy is lowered. Therefore, also from this aspect, these peptides find use for eliciting immunity in cancer patients against ELOVL7. Thus, the peptides of the present invention, preferably, peptides having an amino acid sequence selected from among SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 14, 16, 18, 19, 20, 21, 22, 25, 26 and 27.

[0045] In addition to the above-described modifications, the peptides of the present invention can also be linked to other substances, so long as the resulting linked peptide retains the requisite CTL inducibility of the original peptide. Examples of suitable substances include, for example: peptides, lipids, sugar and sugar chains, acetyl groups, natural and synthetic polymers, etc. The peptides can contain modifications such as glycosylation, side chain oxidation, or phosphorylation, etc., provided the modifications do not destroy the biological activity of the original peptide. These kinds of modifications can be performed to confer additional functions (e.g., targeting function,

and delivery function) or to stabilize the polypeptide.

[0046] For example, to increase the in vivo stability of a polypeptide, it is known in the art to introduce D-amino acids, amino acid mimetics or unnatural amino acids; this concept can also be adapted to the present polypeptides. The stability of a polypeptide can be assayed in a number of ways. For instance, peptidases and various biological media, such as human plasma and serum, can be used to test stability (see, e.g., Verhoef et al., Eur J Drug Metab Pharmacokin 1986, 11: 291-302).

[0047] Herein, the peptides of the present invention can also be described as "ELOVL7 peptide(s)" or "ELOVL7 polypeptide(s)".

[0048] III. Preparation of ELOVL7 peptides

The peptides of the 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 invention can be synthesized individually or as longer polypeptides composed of two or more peptides. The peptides can be then be isolated i.e., purified so as to be substantially free of other naturally occurring host cell proteins and fragments thereof, or any other chemical substances.

[0049] 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.

[0050] A peptide of the present invention can be obtained through chemical synthesis based on the selected amino acid sequence. Examples of conventional peptide synthesis methods that can be adapted 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.

[0051] Alternatively, the present peptides can be obtained adapting 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 polynucleotide encoding the objective peptide in an expressible form (e.g., downstream of a regulatory sequence

corresponding to a promoter sequence) is prepared and transformed into a suitable host cell. The host cell is then cultured to produce the peptide of interest. The peptide can also be produced in vitro adopting an in vitro translation system.

[0052] IV. Polynucleotides

The present invention also provides a polynucleotide which encodes any of the aforementioned peptides of the present invention. These include polynucleotides derived from the natural occurring ELOVL7 gene (GenBank Accession No. AB181393, NM_001104558 or NM_024930 (for example, SEQ ID NO: 28)) 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.

[0053] The polynucleotide of the present invention can be composed of DNA, RNA, and derivatives thereof. A DNA is suitably composed of bases such as A, T, C, and G, and T is replaced by U in an RNA.

[0054] The polynucleotide of the present invention can encode multiple peptides of the present invention with or without intervening amino acid sequences in between. For example, the intervening amino acid sequence can provide a cleavage site (e.g., enzyme recognition sequence) of the polynucleotide or the translated peptides. Furthermore, the polynucleotide can include any additional sequences to the coding sequence encoding the peptide of the present invention. For example, the polynucleotide can be a recombinant polynucleotide that includes regulatory sequences required for the expression of the peptide or can be an expression vector (plasmid) with marker genes and such. In general, such recombinant polynucleotides can be prepared by the manipulation of polynucleotides through conventional recombinant techniques using, for example, polymerases and endonucleases.

[0055] Both recombinant and chemical synthesis techniques can be used to produce the

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

[0056] V. Exosomes

The present invention further provides intracellular vesicles called exosomes, which present complexes formed between the peptides of the present invention and HLA antigens on their surface. Exosomes can be prepared, for example using the methods detailed in Japanese Patent Application Kohyo Publications Nos. Hei 11-510507 and WO99/03499, and can be prepared using APCs obtained from patients who are subject to treatment and/or prevention. The exosomes of the present invention can be inoculated as vaccines, in a fashion similar to the peptides of the present invention.

[0057] The type of HLA antigens included in the complexes must match that of the subject requiring treatment and/or prevention. For example, in the Japanese population, HLA-A24, particularly HLA-A2402, is prevalent and therefore would be appropriate for treatment of a Japanese patient. The use of the A24 type that are highly expressed among the Japanese and Caucasian is favorable for obtaining effective results, and subtypes such as A2402 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 and/or addition of 1, 2, or several amino acids can be performed based on the amino acid sequence of the naturally occurring ELOVL7 partial peptide.

[0058] When using the A24 type HLA antigen for the exosome of the present invention, the peptides having a sequence selected from among SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 14, 16, 18, 19, 20, 21, 22, 25, 26 and 27 find use.

[0059] 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 of the present invention, exosomes, or CTLs.

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

[0061] For example, 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 the subjects, APCs that present the peptides of the present invention are induced in the body of the subject. The phrase "inducing APC" includes contacting (stimulating) a cell with the peptides of the present invention, or nucleotides encoding the peptides of the present invention to present complexes formed between HLA antigens and the peptides of the present invention on cell's surface. 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 the peptide of the present invention.

[0062] 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 with the APCs of step a, with the peptide and

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

[0063] The first subject and the second subject can be the same individual, or may be different individuals. Alternatively, according to the present invention, use of the peptides of the present invention for manufacturing a pharmaceutical composition inducing antigen-presenting cells is provided. In addition, the present invention provides a method or process for manufacturing a pharmaceutical composition inducing antigen-presenting cells. Further, the present invention also provides the peptides of the present invention for inducing antigen-presenting cells. The APCs obtained by step b can be a vaccine for treating and/or preventing cancer, such as bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, NSCLC, prostate cancer, renal carcinoma and SCLC.

[0064] According to an aspect of the present invention, the APCs have a high level of CTL inducibility. In the term of "high level of CTL inducibility", the high level is relative to the level of that by APC contacting with no peptide or peptides which can not induce

the CTL. Such APCs having a high level of CTL inducibility can be prepared by a method which includes the step of transferring a polynucleotide encoding the peptide of the present invention to APCs in vitro as well as the method mentioned above. The introduced genes can be in the form of DNAs or RNAs. Examples of methods for introduction include, without particular limitations, various methods conventionally performed in this field, such as lipofection, electroporation, and calcium phosphate method can be used. More specifically, it can be performed as described in Cancer Res 1996, 56: 5672-7; J Immunol 1998, 161: 5607-13; J Exp Med 1996, 184: 465-72; Published Japanese Translation of International Publication No. 2000-509281. By transferring the gene into APCs, the gene undergoes transcription, translation, and such in the cell, and then the obtained protein is processed by MHC Class I or Class II, and proceeds through a presentation pathway to present partial peptides.

[0065] VII. Cytotoxic T lymphocytes (CTLs)

A CTL induced against any 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 of the present peptides.

[0066] Such CTLs can be obtained by (1) administering the peptides of the present invention to a subject or (2) contacting (stimulating) subject-derived APCs, and CD8-positive cells, or peripheral blood mononuclear leukocytes in vitro with the peptides of the present invention or (3) contacting CD8-positive cells or peripheral blood mononuclear leukocytes in vitro with the APCs or exosomes presenting a complex of an HLA antigen and the peptides on its surface or (4) introducing a gene that includes a polynucleotide encoding a T cell receptor (TCR) subunit biding to the peptide of the present invention. Such APCs or exosomes can be prepared by the methods described above and details of the method of (4) is described bellow in section "VIII. T cell receptor (TCR)".

[0067] 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 of the present invention or exosomes 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 ELOVL7, such as cancer cells, or cells that are transfected with the ELOVL7 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.

[0068] VIII. T cell receptor (TCR)

The present invention also provides a composition including nucleic acids encoding

polypeptides that are capable of forming a subunit of a T cell receptor (TCR), and methods of using the same. The TCR subunits have the ability to form TCRs that confer specificity to T cells against tumor cells presenting ELOVL7. By using the known methods in the art, the nucleic acids of alpha- and beta- chains as 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: 30) and

3-TRa-C primers (5'-tcagctggaccacagccgcagcgt-3') specific to TCR alpha chain C region (SEQ ID NO: 31),

3-TRb-C1 primers (5'-tcagaaatcctttctcttgac-3') specific to TCR beta chain C1 region (SEQ ID NO: 32) or

3-TRbeta-C2 primers (5'- ctagcctctggaatcctttctctt-3') specific to TCR beta chain C2 region (SEQ ID NO: 33) as 3' side primers, but not limited.

The derivative TCRs can bind target cells displaying the ELOVL7 peptide with high avidity, and optionally mediate efficient killing of target cells presenting the ELOVL7 peptide in vivo and in vitro.

[0069] The nucleic acids encoding the TCR subunits can be incorporated into suitable vectors, e.g., retroviral vectors. These vectors are well known in the art. The nucleic acids or the vectors including them usefully can be transferred into a T cell, for example, a T cell from a patient. Advantageously, the invention provides an off-the-shelf composition allowing rapid modification of a patient's own T cells (or those of another mammal) to rapidly and easily produce modified T cells having excellent cancer cell killing properties.

[0070] The specific TCR is a receptor capable of specifically recognizing a complex of a peptide of the present invention and HLA molecule, giving a T cell specific activity against the target cell when the TCR on the surface of the T cell. A specific recognition of the above complex may be confirmed by any known methods, and preferred methods include, for example, tetramer analysis using HLA molecule and peptide of the 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.

[0071] Also, the present invention provides CTLs which are prepared by transduction with

the nucleic acids encoding the TCR subunits polypeptides that bind to the ELOVL7 peptide, e.g., SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 14, 16, 18, 19, 20, 21, 22, 25, 26 and 27 in the context of HLA-A24. 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 invention can be used to form an immunogenic composition useful in treating or the prevention of cancer in a patient in need of therapy or protection (See WO2006/031221, the contents of which are incorporated by reference herein).

[0072] Prevention and prophylaxis include any activity which reduces the burden of mortality or morbidity from disease. Prevention and prophylaxis can occur "at primary, secondary and tertiary prevention levels." While primary prevention and prophylaxis avoid the development of a disease, secondary and tertiary levels of prevention and prophylaxis encompass activities aimed at the prevention and prophylaxis of the progression of a disease and the emergence of symptoms as well as reducing the negative impact of an already established disease by restoring function and reducing disease-related complications. Alternatively, prevention and prophylaxis include a wide range of prophylactic therapies aimed at alleviating the severity of the particular disorder, e.g. reducing the proliferation and metastasis of tumors, reducing angiogenesis.

[0073] Treating and/or for the prophylaxis of cancer or , and/or the prevention of postoperative recurrence thereof includes any of the following steps, such as surgical removal of cancer cells, inhibition of the growth of cancerous cells, involution or regression of a tumor, induction of remission and suppression of occurrence of cancer, tumor regression, and reduction or inhibition of metastasis. Effectively treating and/or the prophylaxis of cancer decreases mortality and improves the prognosis of individuals having cancer, decreases the levels of tumor markers in the blood, and alleviates detectable symptoms accompanying cancer. For example, reduction or improvement of symptoms constitutes effectively treating and/or the prophylaxis include 10%, 20%, 30% or more reduction, or stable disease.

[0074] IX. Pharmaceutical agents

Since ELOVL7 expression is specifically elevated in cancer such as bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, NSCLC, prostate cancer, renal carcinoma and SCLC as compared with normal tissue (Silva et al., Neoplasia 2005 Apr;7(4):348-55), the peptides of the present invention or polynucleotides encoding such peptides can be used for treating and/or for the prophylaxis of cancer, and/or prevention of postoperative recurrence thereof. Thus, the present invention provides a pharmaceutical agent for the treatment and/or prophylaxis of cancer, and/or for the prevention of postoperative recurrence thereof, which includes one or more of the peptides, or polynucleotides of the present invention as an active in-

gredient. Alternatively, the present peptides can be expressed on the surface of any of the foregoing exosomes or cells, such as APCs for the use as pharmaceutical agents. In addition, the aforementioned CTLs which target any of the peptides of the invention can also be used as the active ingredient of the present pharmaceutical agents and compositions.

[0075] In another embodiment, the present invention also provides the use of an active ingredient selected from among:

(a) a peptide of the present invention,

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

(c) an APC of the present invention, and

(d) a cytotoxic T cells of the present invention

in manufacturing a pharmaceutical composition or agent for treating cancer.

[0076] Alternatively, the present invention further provides an active ingredient selected from among:

(a) a peptide of the present invention,

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

(c) an APC of the present invention, and

(d) a cytotoxic T cells of the present invention

for use in for treating cancer.

[0077] Alternatively, the present invention further provides a method or process for manufacturing a pharmaceutical composition or agent for treating cancer, wherein the method or process includes the step of formulating a pharmaceutically or physiologically acceptable carrier with an active ingredient selected from among:

(a) a peptide of the present invention,

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

(c) an APC of the present invention, and

(d) a cytotoxic T cells of the present invention

as active ingredients.

[0078] In another embodiment, the present invention also provides a method or process for manufacturing a pharmaceutical composition or agent for treating cancer, wherein the method or process includes the step of admixing an active ingredient with a pharmaceutically or physiologically acceptable carrier, wherein the active ingredient is selected from among:

(a) a peptide of the present invention,

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

(c) an APC of the present invention, and

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

[0079] Alternatively, the pharmaceutical composition or agent of the present invention may

be used for either or both the prophylaxis of cancer and prevention of postoperative recurrence thereof.

[0080] The pharmaceutical agents and composition 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 a substance that has the function to induce anti-tumor immunity upon inoculation into animals.

[0081] The pharmaceutical agents of the present invention can be used to treat and/or prevent cancers, and/or prevention of postoperative recurrence thereof in subjects or patients including human and any other mammal including, but not limited to, mouse, rat, guinea-pig, rabbit, cat, dog, sheep, goat, pig, cattle, horse, monkey, baboon, and chimpanzee, particularly a commercially important animal or a domesticated animal.

[0082] According to the present invention, peptides having an amino acid sequence selected from among SEQ ID NO: 3, 4, 5, 6, 7, 8, 9, 14, 16, 18, 19, 20, 21, 22, 25, 26 and 27 have been found to be HLA-A24 restricted epitope peptides or the candidates that can induce potent and specific immune response. Therefore, the present pharmaceutical agents which include any of these peptides with the amino acid sequences of SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 14, 16, 18, 19, 20, 21, 22, 25, 26 and 27 are particularly suited for the administration to subjects whose HLA antigen is HLA-A24. The same applies to pharmaceutical agents which contain polynucleotides encoding any of these peptides (i.e. the polynucleotides of the present invention).

[0083] Cancers to be treated by the pharmaceutical agents of the present invention are not limited and include all kinds of cancers wherein ELOVL7 is involved, for example, bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, NSCLC, prostate cancer, renal carcinoma and SCLC.

[0084] The present pharmaceutical agents can contain in addition to the aforementioned active ingredients, other peptides which have the ability to induce CTLs against cancerous cells, other polynucleotides encoding the other peptides, other cells that present the other peptides, or such. Herein, the other peptides that have the ability to induce CTLs against cancerous cells are exemplified by cancer specific antigens (e.g., identified TAAs), but are not limited thereto.

[0085] If needed, the pharmaceutical agents of the present invention can optionally include other therapeutic substances as an active ingredient, so long as the substance does not inhibit the antitumoral effect of the active ingredient, e.g., any of the present peptides. For example, formulations can include anti-inflammatory agents, pain killers, chemotherapeutics, and the like. In addition to including other therapeutic substances in the medicament itself, the medicaments of the present invention can also be administered sequentially or concurrently with the one or more other pharmacologic agents. The amounts of medicament and pharmacologic agent depend, for example, on

what type of pharmacologic agent(s) is/are used, the disease being treated, and the scheduling and routes of administration.

[0086] It should be understood that, in addition to the ingredients particularly mentioned herein, the pharmaceutical agents of the present invention can include other agents conventional in the art having regard to the type of formulation in question.

[0087] In one embodiment of the present invention, the present pharmaceutical agents can be included in articles of manufacture and kits containing materials useful for treating the pathological conditions of the disease to be treated, e.g., cancer. The article of manufacture can include a container of any of the present pharmaceutical agents with a label. Suitable containers include bottles, vials, and test tubes. The containers can be formed from a variety of materials, such as glass or plastic. The label on the container should indicate the agent 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.

[0088] In addition to the container described above, a kit including a pharmaceutical 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.

[0089] The pharmaceutical compositions can, if desired, be presented in a pack or dispenser device which can contain one or more unit dosage forms containing the active ingredient. The pack can, for example, include metal or plastic foil, such as a blister pack. The pack or dispenser device can be accompanied by instructions for administration.

[0090] In another embodiment, the present invention also provides the use of the peptides, and/or polynucleotides of the present invention in manufacturing a pharmaceutical agent for the treatment and/or prophylaxis of (i.e., preventing) cancers (tumors), and/or for prevention of postoperative recurrence thereof. For example, the present invention relates to a use of the peptides having an amino acid sequence selected from among SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 14, 16, 18, 19, 20, 21, 22, 25, 26 and 27, and/or a polynucleotide encoding thereof, for manufacturing a pharmaceutical agent for the treatment and/or prophylaxis of (i.e., preventing) cancers (tumors), and/or for the prevention of postoperative recurrence thereof.

[0091] In a further embodiment, the present invention provides peptides, and/or polynucleotides of the present invention for the use in the treatment and/or prophylaxis of (i.e., preventing) cancers (tumors), and/or for the prevention of postoperative recurrence thereof.

[0092] In yet a further embodiment, the present invention provides a method or process for manufacturing a pharmaceutical agent for the treatment and/or prophylaxis of (i.e.,

preventing) cancers (tumors), and/or for the prevention of postoperative recurrence thereof, wherein the method or process includes step for formulating a pharmaceutically or physiologically acceptable carrier with the peptides, and/or polynucleotides of the present invention as active ingredients.

[0093] In yet another embodiment, the present invention provides a method or process for manufacturing a pharmaceutical agent or composition for the treatment and/or prophylaxis of (i.e., preventing) cancers (tumors), and/or for the prevention of postoperative recurrence thereof, wherein the method or process includes the step of admixing an active ingredient with a pharmaceutically or physiologically acceptable carrier, wherein the active ingredient is the peptides, and/or polynucleotides of the present invention.

[0094] (1) Pharmaceutical agents containing the peptides as the active ingredient

The peptides of the present invention can be administered directly as a pharmaceutical agent, or if necessary, that has been 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 are sterilized water, physiological saline, phosphate buffer, culture fluid and such. Furthermore, the pharmaceutical agents can contain as necessary, stabilizers, suspensions, preservatives, surfactants and such. The pharmaceutical agents of the present invention can be used for anticancer purposes.

[0095] The peptides of the present invention can be prepared as a combination composed of two or more of peptides of the invention, to induce CTL in vivo. The peptide combination can take the form of a cocktail or can be conjugated to each other using standard techniques. For example, the peptides can be chemically linked or expressed as a single fusion polypeptide sequence. The peptides in the combination can be the same or different. By administering the peptides of the present invention, the peptides are presented at a high density by the HLA antigens on APCs, then CTLs that specifically react toward the complex formed between the displayed peptide and the HLA antigen are induced. Alternatively, APCs that present any of the peptides of the present invention on their cell surface are obtained by removing APCs (e.g., DCs) from the subjects, which are stimulated by the peptides of the present invention, CTL is induced in the subjects by readministering these APCs (e.g., DCs) to the subjects, and as a result, aggressiveness towards the cancer cells can be increased.

[0096] The pharmaceutical agents or compositions for the treatment and/or prevention of cancer, which include a peptide of the present invention as the active ingredient, can also include an adjuvant known to effectively establish cellular immunity. Alternatively, the pharmaceutical agents or compositions can be administered with other

active ingredients, or administered by formulation into granules. An adjuvant refers to a compound that enhances the immune response against the protein when administered together (or successively) with the protein having immunological activity. Adjuvants contemplated herein include those described in the literature (Clin Microbiol Rev 1994, 7: 277-89). Examples of suitable adjuvants include, but are not limited to, aluminum phosphate, aluminum hydroxide, alum, cholera toxin, salmonella toxin, and the like.

[0097] 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.

[0098] In another embodiment of the present invention, the peptides of the present invention may also be administered in the form of a pharmaceutically acceptable salt. Examples of preferred salts include salts with an alkali metal, salts with a metal, salts with an organic base, salts with an organic acid and salts with an inorganic acid.

[0099] In some embodiments, the pharmaceutical agents of the invention may further include a component which primes CTL. Lipids have been identified as agents capable of priming CTL in vivo against viral antigens. For example, palmitic acid residues can be attached to the epsilon -and alpha-amino groups of a lysine residue and then linked to a peptide of the invention. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant. As another example of lipid priming of CTL responses, E. coli lipoproteins, such as tripalmitoyl-S-glycerylcysteinlyseryl- serine (P3CSS) can be used to prime CTL when covalently attached to an appropriate peptide (see, e.g., Deres et al., Nature 1989, 342: 561-4).

[0100] The method of administration can be oral, intradermal, subcutaneous, intravenous injection, or such, and systemic administration or local administration to the vicinity of the targeted sites. The administration can be performed by single administration or boosted by multiple administrations. The dose of the peptides of 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, 0.001 mg to 1000 mg, for example, 0.1 mg to 10 mg, and can be administered once in a few days to few months. One skilled in the art can appropriately select a suitable dose.

[0101] (2) Pharmaceutical agents containing polynucleotides as the active ingredient

The pharmaceutical agents of the invention can also contain nucleic acids encoding the peptides disclosed herein in an expressible form. Herein, the phrase "in an expressible form" means that the polynucleotide, when introduced into a cell, will be expressed in vivo as a polypeptide that induces anti-tumor immunity. In an exemplified

embodiment, the nucleic acid sequence of the polynucleotide of interest includes regulatory elements necessary for expression of the polynucleotide. The polynucleotide(s) can be equipped so to achieve stable insertion into the genome of the target cell (see, e.g., Thomas KR & Capecchi MR, Cell 1987, 51: 503-12 for a description of homologous recombination cassette vectors). See, e.g., Wolff et al., Science 1990, 247: 1465-8; U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; and WO 98/04720. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivacaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (see, e.g., U.S. Patent No. 5,922,687).

[0102] 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.

[0103] Delivery of a polynucleotide into a patient can be either 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.

[0104] For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 1993, 12: 488-505; Wu and Wu, Biotherapy 1991, 3: 87-95; Tolstoshev, Ann Rev Pharmacol Toxicol 1993, 33: 573-96; Mulligan, Science 1993, 260: 926-32; Morgan & Anderson, Ann Rev Biochem 1993, 62: 191-217; Trends in Biotechnology 1993, 11(5): 155-215). Methods commonly known in the art of recombinant DNA technology which can also be used for the present invention are described in eds. Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, NY, 1993; and Krieger, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY, 1990.

[0105] The method of administration can be oral, intradermal, subcutaneous, intravenous

injection, or such, and systemic administration or local administration to the vicinity of the targeted sites finds use. The administration can be performed by single administration or boosted by multiple administrations. The dose of the polynucleotide in the suitable carrier or cells transformed with the polynucleotide encoding the peptides of 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, 0.001 mg to 1000 mg, for example, 0.1 mg to 10 mg, and can be administered once every a few days to once every few months. One skilled in the art can appropriately select the suitable dose.

[0106] X. Methods using the peptides, exosomes, APCs and CTLs

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

[0107] (1) Method of inducing antigen-presenting cells (APCs)

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

[0108] 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 contacting APCs with the peptides ex vivo can include steps of:

a: collecting APCs from a subject:, and

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

[0109] 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 proteinaceous 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 peptides of the present invention can be used by themselves or with other peptides of the present invention.

[0110] On the other hands, when the peptides of the present invention are administered to a subject, the APCs are contacted with the peptides in vivo, consequently, the APCs with high CTL inducibility are induced in the body of the subject. Thus, the present invention includes administering the peptides of the present invention to a subject. Similarly, when the polynucleotides of the present invention are administered to a subject in an expressible form, the peptides of this invention are expressed and

contacted with APCs in vivo, consequently, the APCs with high CTL inducibility are induced in the body of the subject. Thus, the present invention also includes administering the polynucleotides of the present invention to a subject. "Expressible form" was described above in section "IX. Pharmaceutical agents (2) Pharmaceutical agents containing polynucleotides as the active ingredient"

[0111] The present invention also includes introducing the polynucleotide of the present invention into an APCs to induce APCs with CTL inducibility. For example, the method can include steps of:

a: collecting APCs from a subject:, and

b: introducing a polynucleotide encoding peptide of the present invention.

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

[0112] (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.

[0113] When the peptides, the polynucleotides, APCs, or exosomes of the present invention are administered to a subject, CTL is induced in the body of the subject, and the strength of the immune response targeting the cancer cells is enhanced. Thus, the methods of the present invention include the step of administering the peptides, the polynucleotides, the APCs or exosomes of the present invention to a subject.

[0114] Alternatively, CTL can be also induced by using them ex vivo, and after inducing CTL, the activated CTLs are returned to the subject. For example, the method can include steps of:

a: collecting APCs from subject:,

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

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

[0115] The APCs to be co-cultured with the CD8-positive cells in above step c can also be prepared by transferring a gene that includes a polynucleotide of the present invention into APCs as described above in section "VI. Antigen-presenting cells"; but are not limited thereto and any APCs which effectively presents the present on its surface a complex of an HLA antigen and the peptide of the present invention can be used for the present method.

[0116] Instead of such APCs, the exosomes that presents on its surface a complex of an HLA antigen and the peptide of the present invention can be also used. Namely, the present invention can includes the step of co-culturing exosomes presenting on its surface a complex of an HLA antigen and the peptide of the present invention. Such exosomes can be prepared by the methods described above in section "V. Exosomes".

[0117] Furthermore, CTL can be induced by introducing a gene that includes a polynu-

cleotide encoding the TCR subunit binding to the peptide of the present invention into CD8-positive cells. Such transduction can be performed as described above in section "VIII. T cell receptor (TCR)".

[0118] (3) Method of inducing immune response

The present invention further provides methods for inducing an immune response against cancer, such as bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, NSCLC, prostate cancer, renal carcinoma and SCLC, in a subject. The methods include the administration of a vaccine one the present invention, which comprises:

(a) one or more epitope peptides of the present invention, or an immunologically active fragment thereof;

(b) one or more polynucleotides encoding the epitope peptides or the immunologically active fragment of (a);

(c) one or more isolated CTLs of the present invention; or

(d) one or more isolated antigen-presenting cells of the present invention.

[0119] In the present invention, cancer overexpressing ELOVL7 can be treated with these active ingredients. The cancer includes, but is not limited to, bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, non-small cell lung cancer (NSCLC), prostate cancer, renal carcinoma and small cell lung cancer (SCLC). Accordingly, prior to the administration of the vaccines or pharmaceutical compositions comprising the active ingredients, it is preferable to confirm whether the expression level of ELOVL7 in the cancer cells or tissues to be treated is enhanced compared with normal cells of the same organ. Thus, in one embodiment, the present invention provides a method for treating cancer (over) expressing ELOVL7, which method may include the steps of:

i) determining the expression level of ELOVL7 in cancer cells or tissue obtained from a subject with the cancer to be treated;

ii) comparing the expression level of ELOVL7 with normal control; and

iii) administrating at least one component selected from the group consisting of (a) to (d) described above to a subject with cancer overexpressing ELOVL7 compared with normal control. Alternatively, the present invention also provides a vaccine or pharmaceutical composition comprising at least one component selected from the group consisting of (a) to (d) described above, for use in administrating to a subject having cancer overexpressing ELOVL7. In other words, the present invention further provides a method for identifying a subject to be treated with the ELOVL7 polypeptide of the present invention, which method may include the step of determining an expression level of ELOVL7 in subject-derived cancer cells or tissue, wherein an increase of the level compared to a normal control level of the gene indicates that the subject has

cancer which may be treated with the ELOVL7 polypeptide of the present invention. The method of treating cancer of the present invention will be described in more detail below.

[0120] A subject to be treated by the present method is preferably a mammal. Exemplary mammals include, but are not limited to, e.g., human, non-human primate, mouse, rat, dog, cat, horse, and cow.

[0121] According to the present invention, the expression level of ELOVL7 in the cancer cells or tissues obtained from a subject is determined. The expression level can be determined at the transcription (nucleic acid) product level, using methods known in the art. For example, the mRNA of ELOVL7 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 ELOVL7. Those skilled in the art can prepare such probes utilizing the sequence information of ELOVL7. For example, the cDNA of ELOVL7 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 the gene may be detected as the intensity of the hybridized labels.

[0122] Furthermore, the transcription product of ELOVL7 (e.g., SEQ ID NO: 28) may be quantified using primers by amplification-based detection methods (e.g., RT-PCR). Such primers can also be prepared based on the available sequence information of the gene.

[0123] Specifically, a probe or primer used for the present method hybridizes under stringent, moderately stringent, or low stringent conditions to the mRNA of ELOVL7. 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 agents, such as formamide.

[0124] Alternatively, the translation product may be detected for the diagnosis of the present invention. For example, the quantity of ELOVL7 protein (SEQ ID NO: 29) may be determined. Methods for determining the quantity of the protein as the translation product include immunoassay methods that use an antibody specifically recognizing the 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 ELOVL7 protein. Methods to prepare these kinds of antibodies for the detection of proteins are well known in the art, and any method may be employed in the present invention to prepare such antibodies and equivalents thereof.

[0125] As another method to detect the expression level of ELOVL7 gene based on its translation product, the intensity of staining may be observed via immunohistochemical analysis using an antibody against ELOVL7 protein. Namely,in this measurement, strong staining indicates increased presence of the protein/level and, at the same time, high expression level of ELOVL7 gene.

[0126] The expression level of a target gene, e.g., including ELOVL7 gene, in cancer cells can be determined to be increased if the level increases from the control level (e.g., the level in normal cells) of the corresponding the target gene 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.

[0127] The control level may be determined at the same time with the cancer cells by using a sample(s) previously collected and stored from a subject/subjects whose disease state(s) (cancerous or non-cancerous) is/are known. 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 ELOVL7 gene 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 ELOVL7 gene 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 ELOVL7 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.

[0128] 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".

[0129] When the expression level of ELOVL7 gene is increased as compared to the normal control level or is similar/equivalent to the cancerous control level, the subject may be diagnosed with cancer to be treated.

More specifically, the present invention provides a method of (i) diagnosing whether a subject has the cancer to be treated, and/or (ii) selecting a subject for cancer treatment, which method includes the steps of:

a) determining the expression level of ELOVL7 in cancer cells or tissue(s) obtained from a subject who is suspected to have the cancer to be treated;

b) comparing the expression level of ELOVL7 with a normal control level;

c) diagnosing the subject as having the cancer to be treated, if the expression level of ELOVL7 is increased as compared to the normal control level; and

d) selecting the subject for cancer treatment, if the subject is diagnosed as having the cancer to be treated, in step c).

Alternatively, such a method includes the steps of:

a) determining the expression level of ELOVL7 in cancer cells or tissue(s) obtained from a subject who is suspected to have the cancer to be treated;

b) comparing the expression level of ELOVL7 with a cancerous control level;

c) diagnosing the subject as having the cancer to be treated, if the expression level of ELOVL7 is similar or equivalent to the cancerous control level; and

d) selecting the subject for cancer treatment, if the subject is diagnosed as having the cancer to be treated, in step c).

[0130] The present invention also provides a kit for determining a subject suffering from cancer which can be treated with the ELOVL7 polypeptide of the present invention, which may also be useful in assessing and/or monitoring the efficacy of a cancer immunotherapy. Preferably, the cancer includes, but is not limited to,bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, NSCLC, prostate cancer, renal carcinoma or SCLC. More particularly, the kit preferably includes at least one reagent for detecting the expression of the ELOVL7 gene in a subject-derived cancer cell, which reagent may be selected from the group of:

(a) a reagent for detecting mRNA of the ELOVL7 gene;

(b) a reagent for detecting the ELOVL7 protein; and

(c) a reagent for detecting the biological activity of the ELOVL7 protein.

[0131] Suitable reagents for detecting mRNA of the ELOVL7 gene include nucleic acids

that specifically bind to or identify the ELOVL7 mRNA, such as oligonucleotides which have a complementary sequence to a portion of the ELOVL7 mRNA. These kinds of oligonucleotides are exemplified by primers and probes that are specific to the ELOVL7 mRNA. These kinds of oligonucleotides may be prepared based on methods well known in the art. If needed, the reagent for detecting the ELOVL7 mRNA may be immobilized on a solid matrix. Moreover, more than one reagent for detecting the ELOVL7 mRNA may be included in the kit.

[0132] On the other hand, suitable reagents for detecting the ELOVL7 protein include antibodies to the ELOVL7 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 ELOVL7 protein. Methods to prepare these kinds of antibodies for the detection of proteins 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 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 ELOVL7 protein may be included in the kit.

[0133] The kit may contain more than one of the aforementioned reagents. For example, tissue samples obtained from subjects without cancer or suffering from cancer or not 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.

[0134] In an embodiment of the present invention, when the reagent is a probe against the ELOVL7 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 ELOVL7 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.

[0135] The kit of the present invention may further include a positive control sample or ELOVL7 standard sample. The positive control sample of the present invention may be prepared by collecting ELOVL7 positive samples and then assaying their ELOVL7 levels. Alternatively, a purified ELOVL7 protein or polynucleotide may be added to cells that do not express ELOVL7 to form the positive sample or the ELOVL7 sample. In the present invention, purified ELOVL7 may be a recombinant protein. The ELOVL7 level of the positive control sample is, for example, more than the cut off value.

[0136] The following examples are presented to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention.

Examples

[0137] Materials and Methods

Cell lines

A24 lymphoblastoid cell line (A24LCL) was established by transformation with Epstein-bar virus into HLA-A24 positive human B lymphocyte. COS7, African green monkey kidney cell line, was purchased from ATCC.

[0138] Candidate selection of peptides derived from ELOVL7

9-mer and 10-mer peptides derived from ELOVL7 that bind to HLA-A*2402 and HLA-A*0201 molecules 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)). These peptides were synthesized by Biosynthesis Inc. (Lewisville, TX) according to a standard solid phase synthesis method and purified by reversed phase high performance liquid chromatography (HPLC). The purity (>90%) and the identity of the peptides were determined by analytical HPLC and mass spectrometry analysis, respectively. Peptides were dissolved in dimethylsulfoxide (DMSO) at 20 mg/ml and stored at -80 degrees C.

[0139] In vitro CTL Induction

Monocyte-derived dendritic cells (DCs) were used as antigen-presenting cells (APCs) to induce cytotoxic T lymphocyte (CTL) responses against peptides presented on human leukocyte antigen (HLA). DCs were generated in vitro as described elsewhere (Nakahara S et al., Cancer Res 2003 Jul 15, 63(14): 4112-8). Specifically, peripheral blood mononuclear cells (PBMCs) isolated from a normal volunteer (HLA-A*2402 positive) by Ficoll-Plaque (Pharmacia) solution were separated by adherence to a plastic tissue culture dish (Becton Dickinson) so as to enrich them as

the monocyte fraction. The monocyte-enriched population was cultured in the presence of 1000 U/ml of granulocyte-macrophage colony-stimulating factor (GM-CSF) (R&D System) and 1000 U/ml of interleukin (IL)-4 (R&D System) in AIM-V Medium (Invitrogen) containing 2% heat-inactivated autologous serum (AS). After 7 days of culture, the cytokine-induced DCs were pulsed with 20 micro g/ml of each of the synthesized peptides in the presence of 3 micro g/ml of beta2-microglobulin for 3 hr at 37 degrees C in AIM-V Medium. The generated cells appeared to express DC-associated molecules, such as CD80, CD83, CD86 and HLA class II, on their cell surfaces (data not shown). These peptide-pulsed DCs were then inactivated by iX-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 A24LCL or T2 cells after the 3rd round of peptide stimulation on day 21 (Tanaka H et al., Br J Cancer 2001 Jan 5, 84(1): 94-9; Umano Y et al., Br J Cancer 2001 Apr 20, 84(8): 1052-7; Uchida N et al., Clin Cancer Res 2004 Dec 15, 10(24): 8577-86; Suda T et al., Cancer Sci 2006 May, 97(5): 411-9; Watanabe T et al., Cancer Sci 2005 Aug, 96(8): 498-506).

[0140] 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 (MMC), in the presence of 40 ng/ml of anti-CD3 monoclonal antibody (Pharmingen). One day after initiating the cultures, 120 IU/ml of IL-2 were added to the cultures. The cultures were fed with fresh AIM-V/5% AS medium containing 30 IU/ml of IL-2 on days 5, 8 and 11 (Tanaka H et al., Br J Cancer 2001 Jan 5, 84(1): 94-9; Umano Y et al., Br J Cancer 2001 Apr 20, 84(8): 1052-7; Uchida N et al., Clin Cancer Res 2004 Dec 15, 10(24): 8577-86; Suda T et al., Cancer Sci 2006 May, 97(5): 411-9; Watanabe T et al., Cancer Sci 2005 Aug, 96(8): 498-506).

[0141] Specific CTL activity

To examine specific CTL activity, interferon (IFN)-gamma enzyme-linked immunospot (ELISPOT) assay and IFN-gamma enzyme-linked immunosorbent assay (ELISA) were performed. Specifically, peptide-pulsed A24LCL (1 x 10^4/well) was prepared as stimulator cells. Cultured cells in 48 wells were used as responder cells.

IFN-gamma ELISPOT assay and IFN-gamma ELISA assay were performed under manufacture procedure.

[0142] 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-A24 was amplified by PCR. The PCR-amplified product of Target genes and HLA-A24 were cloned into pIRES vector (Clontech Laboratories, Inc., Cat. No. 631605). The plasmids were transfected into COS7, which is the target genes and HLA-A24-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 target cells (5 X 10^4 cells/ well) for CTL activity assay.

[0143] Results

Enhanced ELOVL7 expression in cancers

The global gene expression profile data obtained from various cancers using cDNA-microarray revealed that ELOVL7 (GenBank Accession No. AB181393, NM_001104558 or NM_024930 (for example, SEQ ID NO: 28) expression was elevated. ELOVL7 expression was validly elevated in 15 out of 33 bladder cancers, 26 out of 68 breast cancers, 9 out of 14 colorectal cancers, 7 out of 18 esophageal cancer, 3 out of 5 gastric cancers, 9 out of 31 NSCLCs, 19 out of 55 prostate cancers, 12 out of 15 renal carcinomas and 11 out of 15 SCLC, as compared with corresponding normal tissue (Table 1).

[0144] [Table 1]

Ratio of cases observed up-regulation of ELOVL7 in cancerous tissue as compared with normal corresponding tissue

Cancers	Ratio
Bladder Cancer	15/33
Breast Cancer	26/68
Colorectal Cancer	9/14
Esophageal Cancer	7/18
Gastric Cancer	3/5
NSCLC	9/31
Prostate Cancer	19/55
Renal Carcinoma	12/15
SCLC	11/15

[0145] Prediction of HLA-A24 binding peptides derived from ELOVL7

Table 2 shows the HLA-A24 binding peptides of ELOVL7 in order of highest binding affinity. Table 2a shows the 9mer peptides and Table 2b shows the 10mer peptides derived from ELOVL7. A total of 29 peptides having potential HLA-A24

binding ability were selected and examined to determine the epitope peptides.

[0146] [Table 2a]

HLA-A24 binding 9mer peptides derived from ELOVL7

Peptide name	Rank	Start Position	Amino Acid sequence	Binding Score	SEQ ID NO.
ELOVL7-A24-9mer	1	199	KYLWWKKYL	600	1
	2	43	FYVYFVTSL	300	2
	3	120	YYFSKFIEL	264	3
	4	244	SYSFMFLLL	240	4
	6	70	TYNFFIVLF	150	5
	7	80	VYMCYEFVM	37.5	6
	8	246	SFMFLLLFL	36	7
	9	248	MFLLLFLHF	21.6	8
	10	124	KFIELLDTI	21.6	9
	11	121	YFSKFIELL	20	10
	12	205	KYLTSLQLV	18	11
	13	232	KYQFPVFAC	15	12
	14	83	CYEFVMSGW	10.5	13
	15	214	QFVIVAIHI	10.5	14

Start position indicates the number of amino acid residue from the N-terminus of ELOVL7.
Binding score is derived from "BIMAS".

[0147] [Table 2b]

HLA-A24 binding 10mer peptides derived from ELOVL7

Peptide name	Rank	Start Position	Amino Acid sequence	Binding Score	SEQ ID NO.
ELOVL7-A24-10mer	1	119	LYYFSKFIEL	264	15
	2	232	KYQFPVFACI	252	16
	3	120	YYFSKFIELL	200	17
	4	184	MYSYYGLSAL	200	18
	5	244	SYSFMFLLLF	100	19
	6	124	KFIELLDTIF	43.2	20
	7	46	YFVTSLGPKL	39.6	21
	8	42	GFYVYFVTSL	20	22
	9	163	KFAAGGLGTF	20	23
	10	226	FFMEDCKYQF	18	24
	11	199	KYLWWKKYLT	15	25
	13	77	LFSVYMCYEF	11	26
	14	70	TYNFFIVLFS	10.5	27

Start position indicates the number of amino acid residue from the N-terminus of ELOVL7.
Binding score is derived from "BIMAS".

[0148] CTL induction with the predicted peptides from ELOVL7 restricted with HLA-A*2402 and establishment for CTL lines stimulated with ELOVL7 derived peptides

CTLs for those peptides derived from ELOVL7 were generated according to the protocols as described in "Materials and Methods". Peptide specific CTL activity was determined by IFN-gamma ELISPOT assay (Figure 1a-q). It showed that well number

#6 stimulated with ELOVL7-A24-9-120 (SEQ ID NO: 3) (a), #5 with ELOVL7-A24-9-244 (SEQ ID NO: 4) (b), #1 with ELOVL7-A24-9-70 (SEQ ID NO: 5) (c), #7 with ELOVL7-A24-9-80 (SEQ ID NO: 6) (d), #5 with ELOVL7-A24-9-246 (SEQ ID NO: 7) (e), #1 with ELOVL7-A24-9-248 (SEQ ID NO: 8) (f), #5 with ELOVL7-A24-9-124 (SEQ ID NO: 9) (g), #2 with ELOVL7-A24-9-214 (SEQ ID NO: 14) (h), #1 and #6 with ELOVL7-A24-10-232 (SEQ ID NO: 16) (i), #2 with ELOVL7-A24-10-184 (SEQ ID NO: 18) (j), #2 with ELOVL7-A24-10-244 (SEQ ID NO: 19) (k), #1 with ELOVL7-A24-10-124 (SEQ ID NO: 20) (l), #2 with ELOVL7-A24-10-46 (SEQ ID NO: 21) (m), #3, #6 and #7 with ELOVL7-A24-10-42 (SEQ ID NO: 22) (n), #5 with ELOVL7-A24-10-199 (SEQ ID NO: 25) (o), #5 with ELOVL7-A24-10-77 (SEQ ID NO: 26) (p) and #4 with ELOVL7-A24-10-70 (SEQ ID NO: 27) (s) demonstrated potent IFN-gamma production as compared to the control wells. Furthermore, the cells in the positive well number #6 with SEQ ID NO: 3, #5 with SEQ ID NO: 4, #1 with SEQ ID NO: 5, #7 with SEQ ID NO: 6, #1 and #6 with SEQ ID NO: 16, #2 with SEQ ID NO: 18, #2 with SEQ ID NO: 19, #2 with SEQ ID NO: 21, #3, #6 and #7 with SEQ ID NO: 22, #5 with SEQ ID NO: 25, #5 with SEQ ID NO: 26 and #4 with SEQ ID NO: 27 were expanded and established CTL lines. CTL activity of those CTL lines was determined by IFN-gamma ELISA assay (Figure 2a-l). It showed that all CTL lines demonstrated potent IFN-gamma production against the target cells pulsed with corresponding peptide as compared to target cells without peptide pulse. On the other hand, no CTL lines could be established by stimulation with other peptides shown in Table 2, despite those peptide had possible binding activity with HLA-A*2402 (data not shown). The results herein demonstrate that 12 peptides derived from ELOVL7 and screened as the peptides could induce potent CTL lines.

[0149] Establishment of CTL clones against ELOVL7 specific peptides

CTL clones were established by limiting dilution from CTL lines as described in "Materials and Methods", and IFN-gamma production from CTL clones against target cells pulsed peptide were determined by IFN-gamma ELISA assay. Potent IFN-gamma productions were determined from CTL clones stimulated with SEQ ID NO: 3 (a), SEQ ID NO: 6 (b), SEQ ID NO: 7 (c), SEQ ID NO: 17 (d), SEQ ID NO: 19 (e), SEQ ID NO: 20 (f), SEQ ID NO: 23 (g) and SEQ ID NO: 28) (h) in Figure 3.

[0150] Specific CTL activity against target cells exogenously expressing ELOVL7 and HLA-A*2402

The established CTL lines raised against these peptides were examined for their ability to recognize target cells that endogenously express ELOVL7 and HLA-A*2402 molecule. Specific CTL activity against COS7 cells which transfected with both the full length of ELOVL7 and HLA-A*2402 molecule gene (a specific model for the

target cells that exogenously express ELOVL7 and HLA-A*2402 gene) was tested using the CTL lines raised by corresponding peptide as the effecter cells. COS7 cells transfected with either full length of ELOVL7 genes or HLA-A* 2402 were prepared as control. In Figure 4, the CTLs stimulated with ELOVL7-A24-9-120 (SEQ ID NO: 3) (a), ELOVL7-A24-9-70 (SEQ ID NO: 5) (b) and ELOVL7-A24-9-80 (SEQ ID NO: 6) (c) showed potent CTL activity against COS7 cells expressing both ELOVL7 and HLA- A* 2402. On the other hand, no significant specific CTL activity was detected against the controls. Thus, these data clearly demonstrated that ELOVL7-A24-9-120 (SEQ ID NO: 3) (a), ELOVL7-A24-9-70 (SEQ ID NO: 5) (b) and ELOVL7-A24-9-80 (SEQ ID NO: 6) (c) was naturally expressed on the target cells with HLA-A*2402 molecule and were recognized by the CTLs. These results indicate that these peptides derived from ELOVL7 may be available to apply the cancer vaccines for patients with ELOVL7 expressing tumors.

[0151] Homology analysis of antigen peptides

The CTLs stimulated with ELOVL7-A24-9-120 (SEQ ID NO: 3), ELOVL7-A24-9-244 (SEQ ID NO: 4), ELOVL7-A24-9-70 (SEQ ID NO: 5), ELOVL7-A24-9-80 (SEQ ID NO: 6), ELOVL7-A24-9-246 (SEQ ID NO: 7), ELOVL7-A24-9-248 (SEQ ID NO: 8), ELOVL7-A24-9-124 (SEQ ID NO: 9), ELOVL7-A24-9-214 (SEQ ID NO: 14), ELOVL7-A24-10-232 (SEQ ID NO: 16), ELOVL7-A24-10-184 (SEQ ID NO: 18), ELOVL7-A24-10-244 (SEQ ID NO: 19), ELOVL7-A24-10-124 (SEQ ID NO: 20), ELOVL7-A24-10-46 (SEQ ID NO: 21), ELOVL7-A24-10-42 (SEQ ID NO: 22), ELOVL7-A24-10-199 (SEQ ID NO: 25), ELOVL7-A24-10-77 (SEQ ID NO: 26) and ELOVL7-A24-10-70 (SEQ ID NO: 27) showed significant and specific CTL activity. This result may be due to the fact that these peptide sequences are homologous to peptides 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 the BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast/blast.cgi) which revealed no sequence with significant homology. The results of homology analyses indicate that the sequences of ELOVL7-A24-9-120 (SEQ ID NO: 3), ELOVL7-A24-9-244 (SEQ ID NO: 4), ELOVL7-A24-9-70 (SEQ ID NO: 5), ELOVL7-A24-9-80 (SEQ ID NO: 6), ELOVL7-A24-9-246 (SEQ ID NO: 7), ELOVL7-A24-9-248 (SEQ ID NO: 8), ELOVL7-A24-9-124 (SEQ ID NO: 9), ELOVL7-A24-9-214 (SEQ ID NO: 14), ELOVL7-A24-10-232 (SEQ ID NO: 16), ELOVL7-A24-10-184 (SEQ ID NO: 18), ELOVL7-A24-10-244 (SEQ ID NO: 19), ELOVL7-A24-10-124 (SEQ ID NO: 20), ELOVL7-A24-10-46 (SEQ ID NO: 21), ELOVL7-A24-10-42 (SEQ ID NO: 22), ELOVL7-A24-10-199 (SEQ ID NO: 25), ELOVL7-A24-10-77 (SEQ ID NO: 26) and ELOVL7-A24-10-70 (SEQ ID NO: 27) are unique. Thus, the possibility that these

molecules will raise unintended immunologic response to some unrelated molecule is extremely low.

[0152] In conclusion, novel HLA-A24 epitope peptide derived from ELOVL7 were identified and demonstrated to be applicable for cancer immunotherapy.

Industrial Applicability

[0153] The present invention describes new TAAs, particularly those derived from ELOVL7 that induce potent and specific anti-tumor immune responses and have applicability to a wide array of cancer types. Such TAAs warrant further development as peptide vaccines against cancers associated with ELOVL7, e.g., bladder cancer, breast cancer, colorectal cancer, esophageal cancer, gastric cancer, NSCLC, prostate cancer, renal carcinoma and SCLC.

[0154] 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 invention and its preferred embodiments. Through routine experimentation, one skilled in the art will readily recognize that various changes and modifications can be made therein without departing from the spirit and scope of the invention, the metes and bounds of which are defined by the appended claims.

Claims

[Claim 1] An isolated peptide binding to an HLA antigen and having cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide consists of SEQ ID NO: 29 or an immunogenic fragment thereof.

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

[Claim 3] The isolated peptide of claim 1 or 2, wherein the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 14, 16, 18, 19, 20, 21, 22, 25, 26 and 27 further wherein the peptide retains HLA binding activity and CTL inducibility of the peptide consisting of SEQ ID NO: 29.

[Claim 4] The isolated peptide of any one of claims 1 to 3, wherein the peptide is a nonapeptide or decapeptide.

[Claim 5] The isolated peptide of claim 4, wherein the peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 4, 5, 6, 7, 8, 9, 14, 16, 18, 19, 20, 21, 22, 25, 26 and 27 wherein 1, 2, or several amino acid(s) are substituted, inserted, deleted, or added, further wherein the peptide retains HLA binding activity and CTL inducibility of the peptide consisting of SEQ ID NO: 29.

[Claim 6] The peptide of claim 5 having one or both of following characteristics:
(a) second amino acid from N-terminus is or is modified to be an amino acid selected from the group consisting of phenylalanine, tyrosine, methionine and tryptophan, and
(b) C-terminal amino acid is or is modified to be an amino acid selected from the group consisting of phenylalanine, leucine, isoleucine, tryptophan and methionine.

[Claim 7] An isolated polynucleotide encoding a peptide of any of claims 1 to 6.

[Claim 8] An agent for inducing CTL, wherein the agent comprises one or more peptide(s) as set forth in any one of claims 1 to 6, or one or more polynucleotide(s) as set forth in claim 7.

[Claim 9] A pharmaceutical agent comprising one or more peptide(s) as set forth in any one of claims 1 to 6, or one or more polynucleotide(s) as set forth in claim 7, in combination with a pharmacologically acceptable carrier, formulated for a purpose selected from the group consisting of:
(i) treatment of cancer,
(ii) prophylaxis of cancer,
(iii) preventing postoperative recurrence of cancer, and
(iv) combinations thereof.

[Claim 10] The pharmaceutical agent of claim 9, formulated for administration to a subject whose HLA antigen is HLA-A24.

[Claim 11] The pharmaceutical agent of claim 10 or 11, formulated for treatment of cancer.

[Claim 12] A method for inducing an antigen-presenting cell (APC) with CTL inducibility comprising a step selected from the group consisting of:
(a) contacting an APC with a peptide as set forth in any one of claims 1 to 6 in vitro, ex vivo or in vivo, and
(b) introducing a polynucleotide encoding a peptide as set forth in any one of claims 1 to 6 into an APC.

[Claim 13] A method for inducing CTL comprising a step selected from the group consisting of:
(a) co-culturing CD8-positive T cells with APCs, which presents on its surface a complex of an HLA antigen and a peptide as set forth in any one of claims 1 to 6,
(b) co-culturing CD8-positive T cells with exosomes, which presents on its surface a complex of an HLA antigen and a peptide of any one of claims 1 to 6, and
(c) introducing a gene that comprises a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide binding to a peptide as set forth in any one of claims 1 to 6 into a T cell.

[Claim 14] An isolated APC that presents on its surface a complex of an HLA antigen and a peptide as set forth in any one of claims 1 to 6.

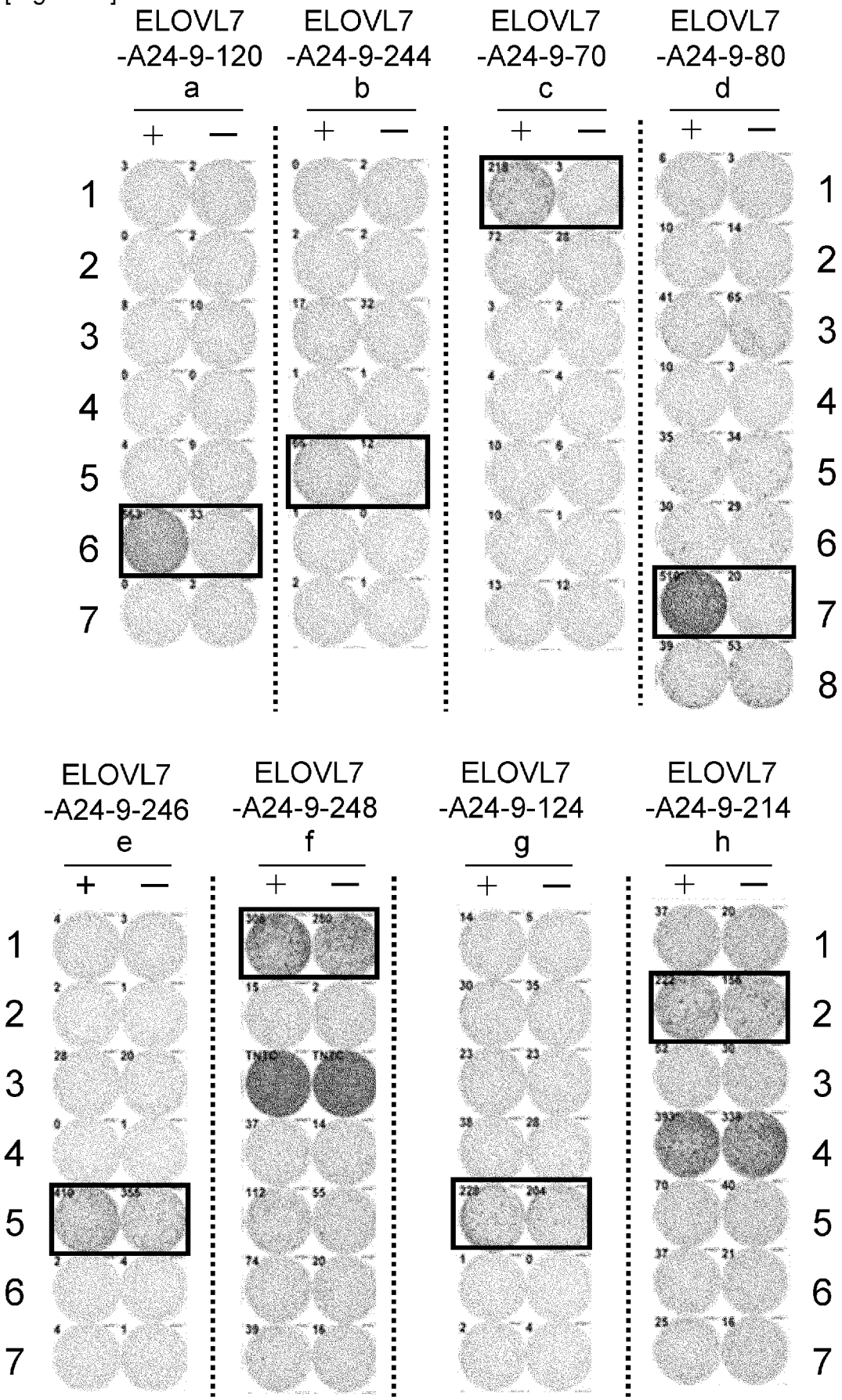
[Claim 15] The APC of claim 14, which is induced by the method of claim 12.

[Claim 16] An isolated CTL that targets the peptide of any one of claims 1 to 6.

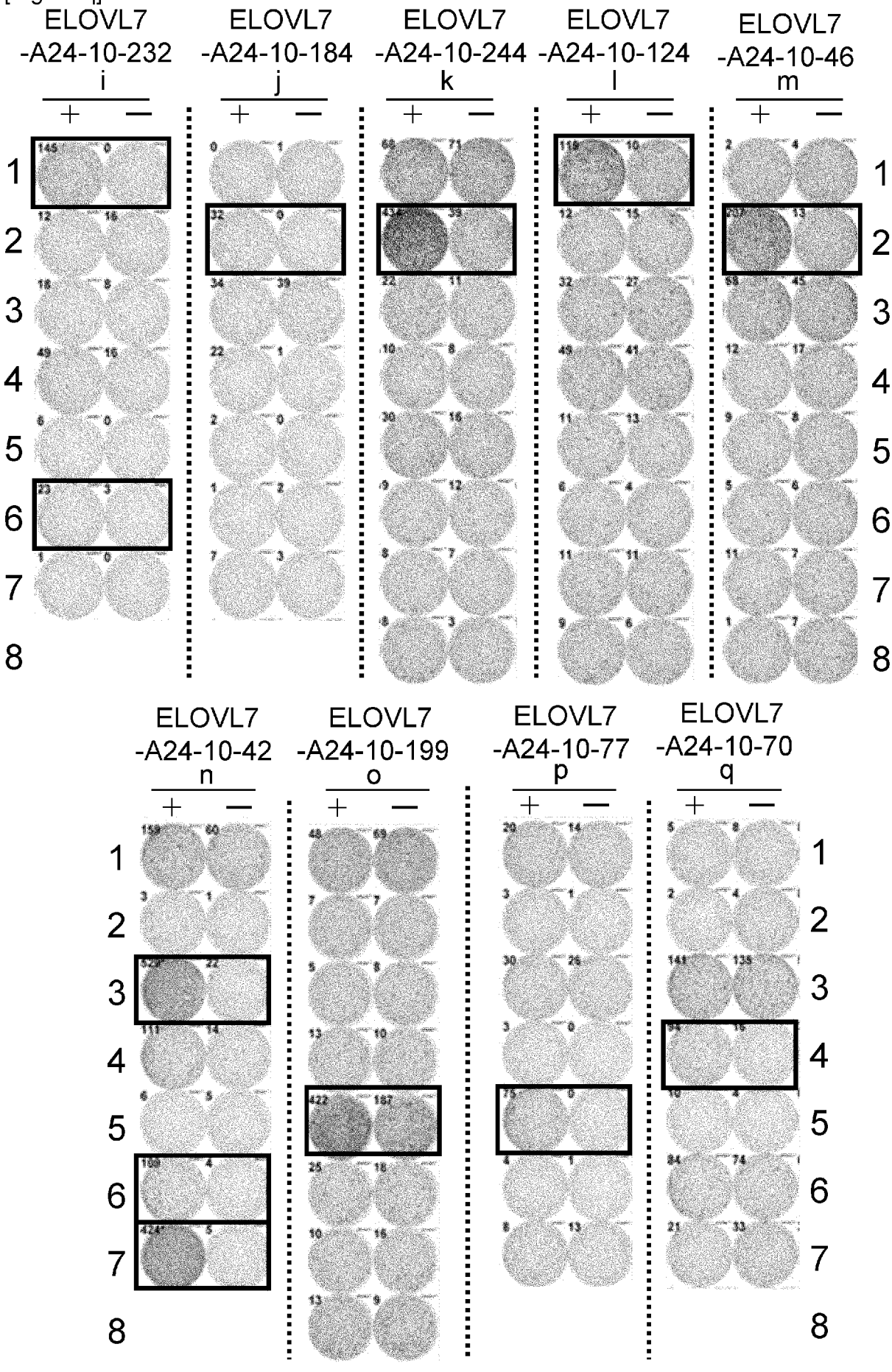
[Claim 17] The CTL of claims 16, which is induced by the method of claim 13.

[Claim 18] A method of inducing immune response against cancer in a subject comprising administering to a subject an agent comprising a peptide as set forth in any one of claims 1 to 6, an immunologically active fragment thereof, or a polynucleotide encoding the peptide or the fragment.

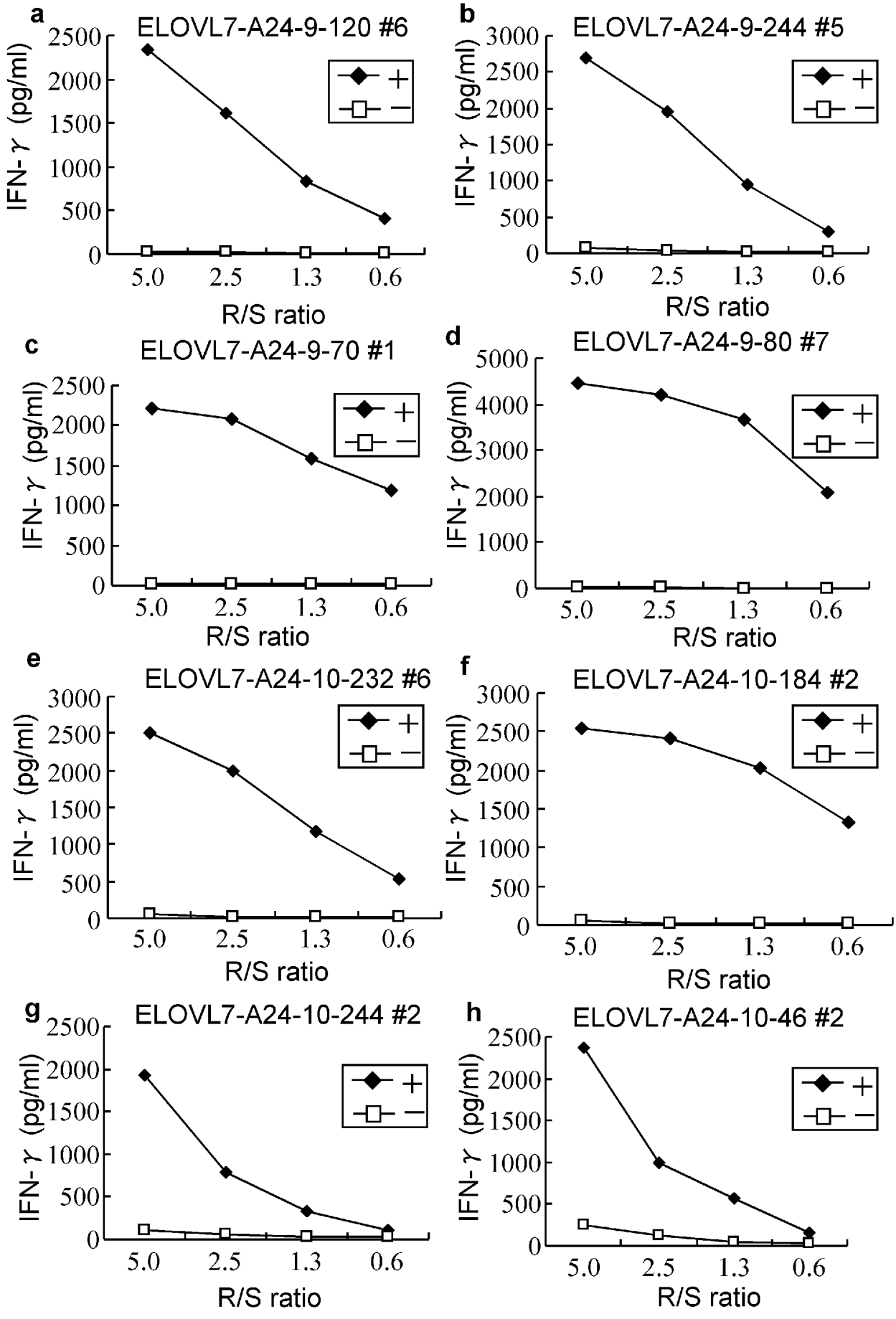
[Fig. 1a-h]


ELOVL7
-A24-9-120
a
ELOVL7
-A24-9-244
b
ELOVL7
-A24-9-70
c
ELOVL7
-A24-9-80
d
ELOVL7
-A24-9-246
e
ELOVL7
-A24-9-248
f
ELOVL7
-A24-9-124
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3
4
5
6
7
8

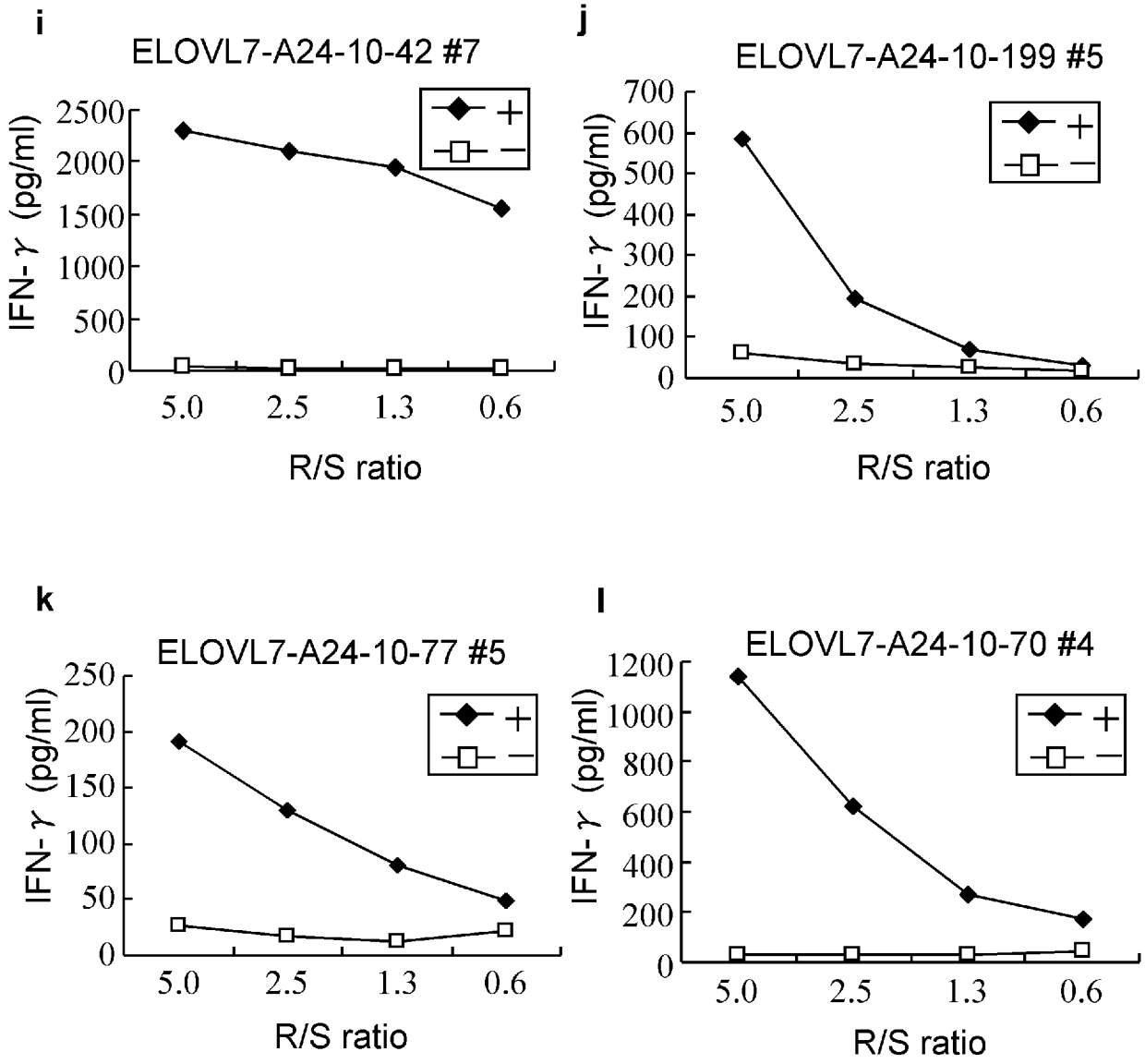
[Fig. 1i-q]


ELOVL7
-A24-10-232
i
ELOVL7
-A24-10-184
j
ELOVL7
-A24-10-244
k
ELOVL7
-A24-10-124
l
ELOVL7
-A24-10-46
m
ELOVL7
-A24-10-42
n
ELOVL7
-A24-10-199
o
ELOVL7
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3
4
5
6
7
8

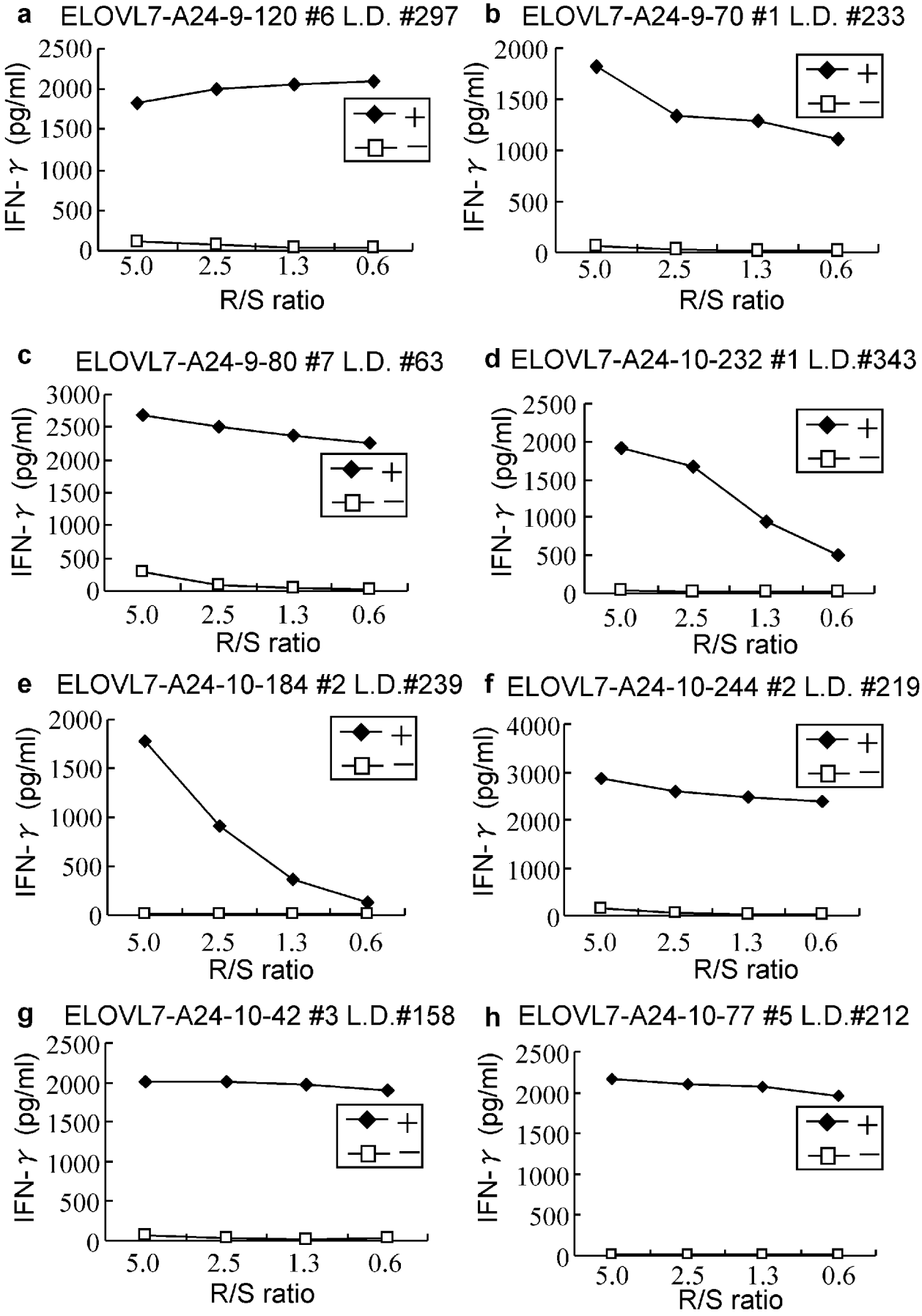
[Fig. 2a-h]


a
ELOVL7-A24-9-120 #6
IFN-γ (pg/ml)
2500
2000
1500
1000
500
0
5.0
2.5
1.3
0.6
R/S ratio
+
−
b
ELOVL7-A24-9-244 #5
3000
c
ELOVL7-A24-9-70 #1
d
ELOVL7-A24-9-80 #7
5000
4000
e
ELOVL7-A24-10-232 #6
f
ELOVL7-A24-10-184 #2
g
ELOVL7-A24-10-244 #2
h
ELOVL7-A24-10-46 #2

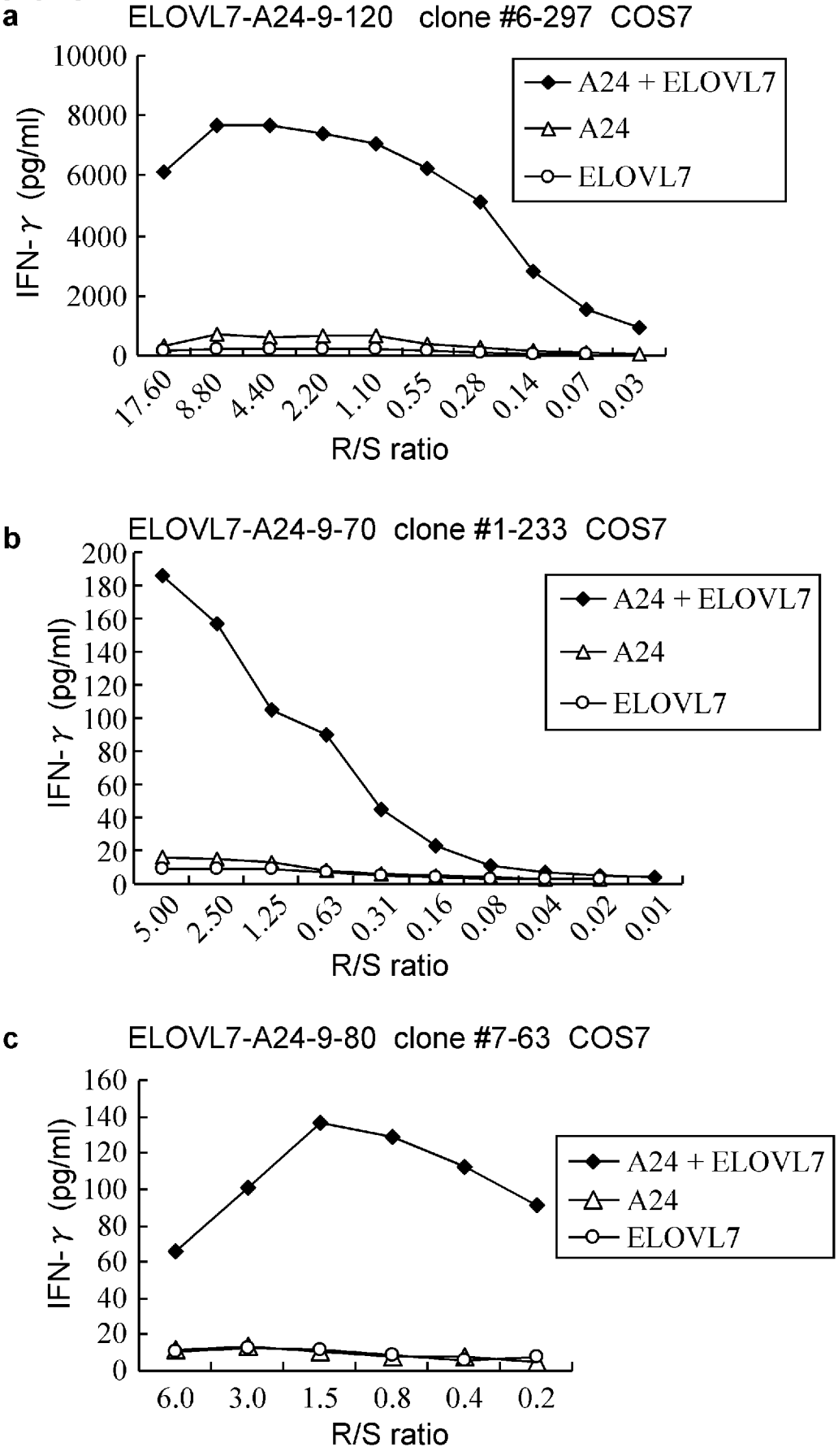
[Fig. 2i-l]


i
ELOVL7-A24-10-42 #7
IFN-γ (pg/ml)
2500
2000
1500
1000
500
0
5.0
2.5
1.3
0.6
R/S ratio
+
−
j
ELOVL7-A24-10-199 #5
IFN-γ (pg/ml)
700
600
500
400
300
200
100
0
5.0
2.5
1.3
0.6
R/S ratio
+
−
k
ELOVL7-A24-10-77 #5
IFN-γ (pg/ml)
250
200
150
100
50
0
5.0
2.5
1.3
0.6
R/S ratio
+
−
l
ELOVL7-A24-10-70 #4
IFN-γ (pg/ml)
1200
1000
800
600
400
200
0
5.0
2.5
1.3
0.6
R/S ratio
+
−

[Fig. 3]


a ELOVL7-A24-9-120 #6 L.D. #297
b ELOVL7-A24-9-70 #1 L.D. #233
c ELOVL7-A24-9-80 #7 L.D. #63
d ELOVL7-A24-10-232 #1 L.D.#343
e ELOVL7-A24-10-184 #2 L.D.#239
f ELOVL7-A24-10-244 #2 L.D. #219
g ELOVL7-A24-10-42 #3 L.D.#158
h ELOVL7-A24-10-77 #5 L.D.#212
IFN-γ (pg/ml)
R/S ratio
5.0
2.5
1.3
0.6
+
−

[Fig. 4]


a
ELOVL7-A24-9-120 clone #6-297 COS7
IFN-γ (pg/ml)
10000
8000
6000
4000
2000
0
A24 + ELOVL7
A24
ELOVL7
17.60
8.80
4.40
2.20
1.10
0.55
0.28
0.14
0.07
0.03
R/S ratio
b
ELOVL7-A24-9-70 clone #1-233 COS7
IFN-γ (pg/ml)
200
180
160
140
120
100
80
60
40
20
0
A24 + ELOVL7
A24
ELOVL7
5.00
2.50
1.25
0.63
0.31
0.16
0.08
0.04
0.02
0.01
R/S ratio
c
ELOVL7-A24-9-80 clone #7-63 COS7
IFN-γ (pg/ml)
160
140
120
100
80
60
40
20
0
A24 + ELOVL7
A24
ELOVL7
6.0
3.0
1.5
0.8
0.4
0.2
R/S ratio

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2009/006869

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. See extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl. C12N15/09, A61K38/00, A61K39/00, A61K48/00, A61P35/00, C07K7/06, C07K14/705, C12N5/07

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2010
Registered utility model specifications of Japan 1996-2010
Published registered utility model applications of Japan 1994-2010

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

GenBank/EMBL/DDBJ/GeneSeq, UniProt/GeneSeq, PubMed, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	"Homo sapiens ELOVL7 mRNA, complete cds.", [online], GenBank, 2006.08.02, ACCESSION: AB181393, [retrieval date: 2010.01.12], <URL: http://www.ncbi.nlm.nih.gov/sviewer/viewer.fcgi?62122556:DDBJ:4812887>	1-3, 7 1-11, 13-17
X	WO 2002/044320 A2 (XENON GENETICS INC.) 2002.06.06, Full text; sequence No.4-5 & JP 2004-513662 A & US 2008/0220500 A1 & EP 1341920 A2	1-3, 7, 9-11
X Y	WO 2007/013479 A2 (ONCOTHERAPY SCIENCE, INC.) 2007.02.01, Full text; sequence No.14-15 & JP 2009-502114 A & EP 1907859 A2 & US 2009-0220512 A1 & CN 101273271 A	1-3, 7, 9-11 1-11, 13-17

☑ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12.01.2010	26.01.2010

Name and mailing address of the ISA/JP	Authorized officer
Japan Patent Office 3-4-3, Kasumigaseki, Chiyoda-ku, Tokyo 100-8915, Japan	NOMURA, Hideo Telephone No. +81-3-3581-1101 Ext. 3448

4B 4155

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2009/006869

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2004/024766 A1 (ONCOTHERAPY SCIENCE, INC.) 2004.03.25, Full text & JP 2007-191485 A & US 2006/0216301 A1 & EP 1548032 A1 & CN 1694901 A	1-11, 13-17
A	WO 2002/064761 A2 (BAYER AG) 2002.08.22, Full text (Family: none)	1-11, 13-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2009/006869

CLASSIFICATION OF SUBJECT MATTER
C12N15/09(2006.01)i, A61K38/00(2006.01)i, A61K39/00(2006.01)i,
A61K48/00(2006.01)i, A61P35/00(2006.01)i, C07K7/06(2006.01)i,
C07K14/705(2006.01)i, C12N5/07(2010.01)i

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2009/006869

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.: 12, 18
because they relate to subject matter not required to be searched by this Authority, namely:
The subject matter of claim 12, 18 relates to a method for treatment of the human or animal body by therapy, which does not require an international search by the International Searching Authority in accordance with PCT Article 17(2)(a)(i) and Rule 39.1(iv).

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
All of the peptides in claim 1 relate to a polypeptide that consists of SEQ ID NO: 29. However, database (cf. ACCESSION: AB181393), WO 2002/044320, WO 2007/013479 have disclosed the polypeptide that consists of SEQ ID NO: 29 of this application. Furthermore, WO 2007/013479 described that the polypeptide was expressed specifically in cancer, and a method for treating or preventing cancer using this polypeptide. Thus, the common technical feature cannot be regarded as a "special technical feature", and there is no other technical relationship among those peptides involving one or more of the same or corresponding technical features. Therefore, these peptides are not so linked as to form a single general inventive concept.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☑ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.