cancer, pancreatic cancer, prostate cancer, renal carcinoma, SCLC and soft tissue tumor. TTK has been identified in bladder cancer, breast cancer, cervical cancer, cholangincellular carcinoma, CML, colorectal cancer, esophageal cancer, liver cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, SCLC and soft tissue tumor. URLC10 has been identified in bladder cancer, cervical cancer, cholangincellular carcinoma, esophageal cancer, gastric cancer, NSCLC, osteosarcoma, pancreatic cancer and SCLC.

[0009] Summary ~~of the Invention~~

The present ~~invention~~disclosure is based in part on the discovery of the applicable targets of immunotherapy. Because TAAs have often no immunogenicity, the discovery of appropriate targets is of extreme importance. As noted above, CDH3, EPHA4, ECT2, HIG2, INHBB, KIF20A, KNTC2, TTK and URLC10 have been identified as up-regulated in various cancers. More particularly, these genes were identified using gene expression profiling with a genome-wide cDNA microarray. As discussed above, expression of CDH3, EPHA4, ECT2, HIG2, INHBB, KIF20A, KNTC2, TTK and URLC10 has been shown to be specifically up-regulated in various tumor cells, from pancreatic cancer cells to renal cell carcinomas. As described in Table 1, CDH3 expression is validly elevated in 26 out of 34 bladder cancer, 17 out of 19 cervical cancer, all of 19 cholangincellular carcinoma, 30 out of 34 colorectal cancer, 20 out of 21 endometriosis, 13 out of 20 gastric cancer, 7 out of 8 diffuse-type gastric cancer, 36 out of 37 NSCLC, all of 16 pancreatic cancer, all of 21 soft tissue tumor and all of 10 testicular tumor.

[0010] Table 1 further demonstrates that:

EPHA4 expression isvalidly elevated in 14 out of 34 bladder cancer, 8 out of 14 cervical cancer, 10 out of 25 cholangincellular carcinoma, 5 out of 15 endometriosis, 5 out of 8 diffuse-type gastric cancer, all of 5 ovarian cancer, all 14 pancreatic cancer, 20 out of 51 prostate cancer and 14 out of 23 soft tissue tumor.

ECT2 expression is validly elevated in 17 out of 19 bladder cancer, 5 out of 12 breast cancer, all of 14 cervical cancer, all of 13 cholangiocellular carcinoma, all of 5 CML, 7 out of 8 colorectal cancer, 12 out of 16 esophageal cancer, 6 out of 16 NSCLC, 8 out of 10 lymphoma, 1 out of 1 pancreatic cancer, 10 out of 13 prostate cancer, 3 out of 6 renal carcinoma and 12 out of 13 SCLC cancer.

HIG2 expression is validly elevated in 19 out of 20 renal cancer and 7 out of 9 soft tissue tumor.

INHBB expression is validly elevated in 10 out of 21 cholangiocellular carcinoma, all of 12 esophageal cancer, 10 out of 13 NSCLC, 22 out of 24 renal carcinoma, 8 out of 14 SCLC cancer and 45 out of 49 soft tissue tumor.

KIF20A expression is validly elevated in all of 31 bladder cancer, 38 out of 61 breast

cancer, 10 out of 11 cholangiocellular carcinoma, 7 out of 19 esophageal cancer, 21 out of 22 NSCLC, all of 6 ovarian cancer, 17 out of 36 prostate cancer, 6 out of 11 renal carcinoma and all of 15 SCLC.

KNTC2 expression is validly elevated in 30 out of 32 bladder cancer, 47 out of 56 breast cancer, all of 10 cervical cancer, 16 out of 22 cholangioncellular carcinoma, 17 out of 37 CML, 3 out of 10 colorectal cancer, 11 out of 46 esophagus cancer, 15 out of 19 NSCLC, 7 out of 8 lymphoma, 20 out of 24 osteosarcoma, 3 out of 5 ovarian cancer, all of 2 pancreatic cancer, 15 out of 37 prostate cancer, 14 out of 19 renal carcinoma, all of 15 SCLC and 40 out of 59 soft tissue tumor.

TTK expression is validly elevated in all of 27 bladder cancer, 25 out of 30 breast cancer, 15 out of 16 cervical cancer, all of 10 cholangiocellular carcinoma, 5 out of 7 CML, 6 out of 10 colorectal cancer, 24 out of 44 esophageal cancer, 8 out of 15 liver cancer, all of 12 NSCLC, all of 6 lymphoma, 13 out of 16 osteoblastoma, 12 out of 17 prostate cancer, all of 15 SCLC and 16 out of 33 soft tissue tumor.

URLC10 expression is validly elevated in all of 29 bladder cancer, 15 out of 16 cervical cancer, all of 7 cholangiocellular carcinoma, 7 out of 19 esophageal cancer, all of 3 gastric cancer, 24 out of 27 NSCLC, 15 out of 19 osteosarcoma, 4 out of 5 pancreatic cancer, 33 out of 43 soft tissue tumor.

[0011] The present ~~invention~~disclosure is based, at least in part, on the identification of specific epitope peptides of the gene products of these genes (CDH3, EPHA4, ECT2, HIG2, INHBB, KIF20A, KNTC2, TTK and URLC10) which possess the ability to induce cytotoxic T lymphocytes (CTLs) specific to the corresponding molecules. As discussed in detail below, Peripheral Blood Mononuclear Cells (PBMC) of healthy donor were stimulated using HLA-A*2402 or HLA-A*0201 binding candidate peptides derived from CDH3, EPHA4, ECT2, HIG2, INHBB, KIF20A, KNTC2, TTK or URLC10. CTL clones and/or lines were then established with specific cytotoxicity against the HLA-A24 or HLA-A2 positive target cells pulsed with each of the candidate peptides. These results demonstrate that these peptides are HLA-A24 or HLA-A2 restricted epitope peptides that can induce potent and specific immune responses against cells expressing CDH3, EPHA4, ECT2, HIG2, INHBB, KIF20A, KNTC2, TTK or URLC10.

[0011a] According to a first aspect, there is provided a peptide of less than 15 amino acids having cytotoxic T cell inducibility, wherein said peptide comprises the amino acid sequence of SEQ ID NO: 194, 174, 178, or 186.

[0011b] According to a second aspect, there is provided a peptide of less than 15 amino acids having cytotoxic T cell inducibility, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 194, 174, 178, and 186 in which 1 or 2 amino acids are substituted, wherein said peptide induces a cytotoxic T cell specific to a cell expressing KIF20A.

[0012] Accordingly, the present ~~invention~~disclosure provides methods for treating or preventing a disease associated with the over-expression of CDH3, EPHA4, ECT2, HIG2, INHBB, KIF20A, KNTC2, TTK or URLC10, e.g. cancer. Such methods involve the step of administering to a subject in need thereof a CDH3, EPHA4, ECT2, HIG2, INHBB, KIF20A, KNTC2, TTK and/or URLC10 polypeptides of the ~~invention~~disclosure. Administration of such peptide(s) results in the induction of anti-tumor immunity is induced by the administration of these polypeptides. Thus, the present ~~invention~~disclosure provides methods for inducing anti-tumor immunity in a subject, such methods involving the step of admin-

istering to the subject the CDH3, EPHA4, ECT2, HIG2, INHBB, KIF20A, KNTC2, TTK and/or URLC10 polypeptides, as well as pharmaceutical compositions for treating or preventing a disease associated with the over-expression of CDH3, EPHA4, ECT2, HIG2, INHBB, KIF20A, KNTC2, TTK and/or URLC10, e.g. cancer, that include the CDH3, EPHA4, ECT2, HIG2, INHBB, KIF20A, KNTC2, TTK and URLC10 polypeptides. Examples of such cancers include, but are not limited to., bladder cancer, breast cancer, cervical cancer, cholangincellular carcinoma, CML, colorectal cancer, endometriosis, esophageal cancer, gastric cancer, diffused type gastric cancer, liver, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma, SCLC, soft tissue tumor and testicular tumor.

[0012a] In a third aspect, there is provided a pharmaceutical composition for use in treating or preventing cancer, said composition comprising at least one peptide as defined above or a polynucleotide encoding the peptide.

[0012b] In a fourth aspect, there is provided an exosome that presents on its surface a complex comprising a peptide as defined above and an HLA antigen.

[0012c] In a fifth aspect, there is provided an in vitro method of inducing an antigen-presenting cell having cytotoxic T cell inducibility, said method comprising the step of:

(a) contacting an antigen-presenting cell with a peptide as defined above, or

(b) transferring a gene comprising a polynucleotide encoding a peptide as defined above to an antigen-presenting cell.

[0012d] In a sixth aspect, there is provided an in vitro method of inducing a cytotoxic T cell by contacting a T cell with a peptide as defined above.

[0012e] In a seventh aspect, there is provided an in vitro method of inducing a cytotoxic T cell, comprising the steps of:

(i) contacting an antigen-presenting cell with a peptide as defined above, and

(ii) mixing the antigen-presenting cell of step (i) with a $CD8^+$ T cell and co-culturing.

[0012f] In an eighth aspect, there is provided an isolated cytotoxic T cell, which is induced by the method as defined above or which is transduced with the nucleic acids encoding the TCR subunits polypeptides binding with a peptide as defined above in the context of HLA-A24.

[0012g] In a ninth aspect, there is provided an antigen-presenting cell, which comprises a complex formed between an HLA antigen and a peptide as defined above, or induced by the method as defined above.

[0012h] In a tenth aspect, there is provided a peptide as defined above, or a polynucleotide encoding said peptide for use in treating or preventing cancer.

[0012i] In an eleventh aspect, there is provided a method of identifying a peptide having an ability to induce a cytotoxic T cell against cells expressing KIF20A, said method comprising the steps of:

(i) providing at least one candidate sequence which consists of an amino acid sequence modified by substituting 1 or two amino acid residues to an original amino acid sequence, wherein the original amino acid sequence is selected from the group consisting of SEQ ID NO: 194, 174, 178, and 186;
(ii) selecting the candidate sequence that does not have substantial significant homology with the peptides derived from any known human gene products other than KIF20A;
(iii) contacting a peptide consisting of a candidate sequence selected in step (ii) with antigen presenting cells;
(iv) contacting the antigen presenting cells of step (iii) with a T-cell to evaluate the ability of the peptide to stimulate the T-cells; and
(v) identifying the peptide of which cytotoxic T cell inducibility is same to or higher than a peptide consisting of the original amino acid sequence.

[0013]
The present ~~invention~~disclosure further provides methods for preventing post-surgery recurrence of the disease mentioned above.
Regarding the specific aims and objectives recited above, it will be understood by those skilled in the art that one or more aspects of this invention can meet certain objectives, while one or more other aspects can meet certain other objectives. Each objective may not apply equally, in all its respects, to every aspect of this invention. As such, the objects herein can be viewed in the alternative with respect to any one aspect of this invention.
[0014]
Additional 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 preferred 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

[0015]

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

CLAIMS

[Claim 1]
An isolated peptide of less than about 15 amino acids selected from the group consisting of peptides comprising the amino acid sequences of SEQ ID NO: 19, 22, 30, 34, 344, 358, 41, 44, 46, 48, 78, 80, 100, 101, 110, 111, 387, 112, 394, 395, 133, 135, 137, 426, 174, 178, 186, 194, 196, 202, 210, 213, 214, 217 or 223.
[Claim 2]
A peptide having cytotoxic T cell inducibility, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: SEQ ID NO: 19, 22, 30, 34, 344, 358, 41, 44, 46, 48, 78, 80, 100, 101, 110, 111, 387, 112, 394, 395, 133, 135, 137, 426, 174, 178, 186, 194, 196, 202, 210, 213, 214, 217 or 223, wherein 1, 2, or several amino acids are substituted, deleted, or added.
[Claim 3]
The peptide of claim 2, wherein the second amino acid from the N-terminus is phenylalanine, tyrosine, methionine, or tryptophan.
[Claim 4]
The peptide of claim 2 or 3, wherein the C-terminal amino acid is phenylalanine, leucine, isoleucine, tryptophan, or methionine.
[Claim 5]
An isolated peptide of less than about 15 amino acids selected from the group consisting of peptides comprising the amino acid sequences of SEQ ID NO: 376, 379, 114, 116, 117, 121, 227, 228, 233, 254, 271, 272 or 288.
[Claim 6]
A peptide having cytotoxic T cell inducibility, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: SEQ ID NO: 376, 379, 114, 116, 117, 121, 227, 228, 233, 254, 271, 272 or 288, wherein 1, 2, or several amino acids are substituted, deleted, or added.
[Claim 7]
The peptide of claim 6, wherein the second amino acid from the N-terminus is leucine or methionine.
[Claim 8]
The peptide of claim 6 or 7, wherein the C-terminal amino acid is valine or leucine.
[Claim 9]
A pharmaceutical composition for treating or preventing a disease associated with over-expression of the genes of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15 and/or 17 said composition comprising one or more peptides of claim 1 to 8 or a polynucleotide encoding the peptides.
[Claim 10]
A pharmaceutical composition of claim 9, wherein the disease associating genes of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15 and/or 17 include cancers.
[Claim 11]
A pharmaceutical composition of claim 10, wherein the cancers include bladder cancer, breast cancer, cervical cancer, cholangincellular carcinoma, CML, colorectal cancer, endometriosis, esophageal cancer, gastric cancer, diffused type gastric cancer, liver cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma, SCLC, soft tissue tumor and

testicular tumor.
[Claim 12]
An exosome that presents on its surface a complex comprising a peptide of claim 1-4 and an HLA antigen.
[Claim 13]
The exosome of claim 12, wherein the HLA antigen is HLA-A24.
[Claim 14]
The exosome of claim 13, wherein the HLA antigen is HLA-A2402.
[Claim 15]
An exosome that presents on its surface a complex comprising a peptide of claim 5-8 and an HLA antigen.
[Claim 16]
The exosome of claim 15, wherein the HLA antigen is HLA-A2.
[Claim 17]
The exosome of claim 16, wherein the HLA antigen is HLA-A0201.
[Claim 18]
A method of inducing antigen-presenting cells having a high cytotoxic T cell inducibility comprising the step of contacting an antigen-presenting cell with a peptide of claim 1-8.
[Claim 19]
A method of inducing cytotoxic T cells by contacting a T cell with a peptide of claim 1-8.
[Claim 20]
A method of inducing antigen-presenting cells having high cytotoxic T cell inducibility, said method comprising the step of transferring a gene comprising a polynucleotide encoding a peptide of claim 1 to 8 to an antigen-presenting cell.
[Claim 21]
An isolated cytotoxic T cell, which is induced by contacting a T cell with a peptide of claim 1-8 or which is transduced with the nucleic acids encoding the TCR subunits polypeptides binding with a peptide of any one claims 1 to 8 in the context of HLA-A24 or HLA-A2.
[Claim 22]
An antigen-presenting cell, which comprises a complex formed between an HLA antigen and a peptide of claim 1 to 8.
[Claim 23]
An antigen-presenting cell, induced by the method of claim 18-20.
[Claim 24]
A vaccine for inhibiting proliferation of cells expressing genes of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15 and/or 17 wherein the vaccine comprises a peptide of claim 1-8 as the active ingredient.
[Claim 25]
The vaccine of claim 24, wherein the cells expressing genes of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15 and/or 17 include cancers.
[Claim 26]
The vaccine of claim 25, wherein the cancers include bladder cancer, breast cancer, cervical cancer, cholangincellular carcinoma, CML, colorectal cancer, endometriosis, esophageal cancer, gastric cancer, diffused type gastric cancer, liver cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma, SCLC, soft tissue tumor and testicular tumor.
[Claim 27]
The vaccine of claim 26, formulated for administration to a subject whose HLA antigen is HLA-A24 or HLA-A2.
[Claim 28]
A method of treating or preventing a disease associated with over-expression of the genes of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15 and/or 17 in a subject comprising admin-

istering to said subject a vaccine comprising a peptide of claim 1-8, an immunologically active fragment thereof, or a polynucleotide encoding said peptide or immunologically active fragment.

[Claim 29]

The method of claim 28, wherein the disease associating genes of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15 and/or 17 include cancers.

[Claim 30]

A method of treating or preventing cancers of claim 29, wherein the cancer cells include bladder cancer, breast cancer, cervical cancer, cholangincellular carcinoma, CML, colorectal cancer, endometriosis, esophageal cancer, gastric cancer, diffused type gastric cancer, liver cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma, SCLC, soft tissue tumor and testicular tumor.

[Claim 31]

A method of screening for a peptide which 1, 2, or several amino acids are substituted, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: SEQ ID NO: 19, 22, 30, 34, 344, 358, 41, 44, 46, 48, 78, 80, 100, 101, 110, 111, 387, 112, 394, 395, 133, 135, 137, 426, 174, 178, 186, 194, 196, 202, 210, 213, 214, 217 or 223, said method comprising the steps of:

(a) comforming no significant sequence homology to the entire sequence of 1, 2 or several amino acids substitute;

(b) measuring the CTL inducibility of the candidate substitute peptide; and

(c) selecting the peptide which CTL inducibility is same to or higher than the original peptide.

CLAIMS

1. A peptide of less than 15 amino acids having cytotoxic T cell inducibility, wherein said peptide comprises the amino acid sequence of SEQ ID NO: 194, 174, 178, or 186.

2. A peptide of less than 15 amino acids having cytotoxic T cell inducibility, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 194, 174, 178, and 186 in which 1 or 2 amino acids are substituted, wherein said peptide induces a cytotoxic T cell specific to a cell expressing KIF20A.

3. The peptide of claim 2, wherein the second amino acid from the N-terminus of the amino acid sequence of SEQ ID NO: 194, 174, 178, or 186 is substituted with phenylalanine, tyrosine, methionine, or tryptophan.

4. The peptide of claim 2 or 3, wherein the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 194, 174, 178, or 186 is substituted with phenylalanine, leucine, isoleucine, tryptophan, or methionine.

5. The peptide of claim 1, wherein said peptide consists of the amino acid sequence of SEQ ID NO: 194, 174, 178, or 186.

6. A pharmaceutical composition for use in treating or preventing cancer, said composition comprising at least one peptide of any one of claims 1 to 5 or a polynucleotide encoding the peptide.

7. An exosome that presents on its surface a complex comprising a peptide of any one of claims 1 to 5 and an HLA antigen.

8. The exosome of claim 7, wherein the HLA antigen is HLA-A24.

9. The exosome of claim 8, wherein the HLA antigen is HLA-A2402.

10. An in vitro method of inducing an antigen-presenting cell having cytotoxic T cell inducibility, said method comprising the step of:

(a) contacting an antigen-presenting cell with a peptide of any one of claims 1 to 5, or
(b) transferring a gene comprising a polynucleotide encoding a peptide of any one of claims 1 to 5 to an antigen-presenting cell.

11. An in vitro method of inducing a cytotoxic T cell by contacting a T cell with a peptide of any one of claims 1 to 5.

12. An in vitro method of inducing a cytotoxic T cell, comprising the steps of:

(i) contacting an antigen-presenting cell with a peptide of any one of claims 1 to 5, and
(ii) mixing the antigen-presenting cell of step (i) with a $CD8^+$ T cell and co-culturing.

13. An isolated cytotoxic T cell, which is induced by the method of claim 11 or 12 or which is transduced with the nucleic acids encoding the TCR subunits polypeptides binding with a peptide of any one of claims 1 to 5 in the context of HLA-A24.

14. An antigen-presenting cell, which comprises a complex formed between an HLA antigen and a peptide of any one of claims 1 to 5, or induced by the method of claim 10.

15. A peptide of any one of claims 1 to 5, or a polynucleotide encoding said peptide for use in treating or preventing cancer.

16. The pharmaceutical composition of claim 6, or the peptide or polynucleotide of claim 15, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, endometriosis, esophageal cancer, gastric cancer, diffused type gastric cancer, liver cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma, SCLC, soft tissue tumor and testicular tumor.

17. A method of identifying a peptide having an ability to induce a cytotoxic T cell against cells expressing KIF20A, said method comprising the steps of:

(i) providing at least one candidate sequence which consists of an amino acid sequence modified by substituting 1 or two amino acid residues to an original amino acid sequence, wherein the original amino acid sequence is selected from the group consisting of SEQ ID NO: 194, 174, 178, and 186;
(ii) selecting the candidate sequence that does not have substantial significant homology with the peptides derived from any known human gene products other than KIF20A;
(iii) contacting a peptide consisting of a candidate sequence selected in step (ii) with antigen presenting cells;
(iv) contacting the antigen presenting cells of step (iii) with a T-cell to evaluate the ability of the peptide to stimulate the T-cells; and
(v) identifying the peptide of which cytotoxic T cell inducibility is same to or higher than a peptide consisting of the original amino acid sequence.