cancers (SCLCs) (PTL1/WO2007/013665) and esophageal cancers (PTL2/WO2007/013671) through analyses of gene-expression profiles using genome-wide cDNA microarrays. These genes have been amply investigated with the hopes of identifying good candidates as immunotherapeutic targets from among them. In order to target cancer cells specifically in immunotherapy, preferred TAAs should be expressed primarily by cancer cells, with limited or no expression by normal healthy tissues.

Preferred TAAs as immunotherapy targets are those that are indispensable for proliferation and survival of cancer cells. Such TAAs may minimize the well-described risk of immune escape of cancer cells attributable to deletion, mutation, or down-regulation of TAAs as a consequence of therapeutically driven immune selection.

[0005] Using gene expression profiling with a genome-wide cDNA microarray containing 23,040 genes, TTK Protein kinase (TTK) was identified as one of the genes up-regulated in lung cancer (NPL3/Kikuchi at al., Int J Oncol. 2006 Apr;28(4):799-805). Expression of TTK is specifically up-regulated in tumor cells in more than 80% of the patients with lung cancer and esophageal cancer. At the same time, TTK is not expressed in any other normal vital organ, except the testis. Taken together, these facts suggest TTK may be applicable as a target of cancer immunotherapy for patient with TTK up-regulated tumors. Peptides derived from TTK that have specific CTL inducibility against target cells exogenously expressing TTK and HLA-A*0201 have previously been disclosed (See WO2008/102557 (PTL3), the results of which are duplicated herein as Figure 4).

Citation List

Patent Literature

[0006] [PTL 1] WO2007/013665
[PTL2] WO2007/013671
[PTL 3] WO2008/102557

Non Patent Literature

[0007] [NPL 1] Boon T, Int J Cancer 1993 May 8, 54(2): 177-80
[NPL 2] Boon T & van der Bruggen P, J Exp Med 1996 Mar 1, 183(3): 725-9
[NPL3] Kikuchi at al., Int J Oncol. 2006 Apr;28(4):799-805

Summary ~~of Invention~~

[0008] The present disclosure~~invention~~ is based, at least in part, on the discovery of novel peptides that may serve as targets of immunotherapy. Because TAAs are sometimes 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, TTK (a typical amino acid sequence and gene sequence are shown in SEQ ID NO: 40 and SEQ

ID NO: 39, respectively, but are not limited to, and a typical gene sequence is also available from, for example, GenBank Accession No. NM_003318) has been identified as up-regulated in cancers, including, but not limited to, lung cancer, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, Chronic myelogenous leukemia (CML), colorectal cancer, endometriosis, esophageal cancer, gastric cancer, diffused type gastric cancer, liver cancer, non-small cell lung cancer (NSCLC), lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, renal carcinoma, small cell lung cancer (SCLC), soft tissue tumor and testicular tumor. Thus, the present invention focuses on TTK as a candidate for the target of cancer/tumor immunotherapy.

[0009] The present disclosure~~invention~~ further relates to the identification of specific epitope peptides of the gene products of TTK that possess the ability to induce CTLs specific to TTK. As discussed in detail below, peripheral blood mononuclear cells (PBMCs) obtained from a healthy donor were stimulated using HLA-A*0201 binding candidate peptides derived from TTK. CTL lines were then established with specific cytotoxicity against the HLA-A2 positive target cells pulsed with each of candidate peptides. Among those CTL lines, CTL lines induced with a peptide having an amino acid sequence of SEQ ID NO: 3 showed significantly potent specific cytotoxicity against cells expressing HLA-A*0201 and TTK. These results demonstrate that these peptides (especially a peptide having an amino acid sequence of SEQ ID NO: 3) are HLA-A2 restricted epitope peptides that may induce potent and specific immune responses against cells expressing TTK. Further, the results indicate that TTK is strongly immunogenic and the epitopes thereof are effective targets for caner/tumor immunotherapy.

[0010] Accordingly, it is an object of the present disclosure~~invention~~ to provide isolated peptides that bind to HLA antigen, particularly those that include an amino acid sequence of TTK (e.g., SEQ ID NO: 40) or an immunologically active fragment thereof. 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 lung cancer, 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. Preferred peptides are peptides having an amino acid sequence of SEQ ID NO: 3.

[0011] The peptides of the present disclosure~~invention~~ encompass those wherein one, two or more amino acids are substituted, deleted and/or added, so long as the resulting modified peptides retain the original CTL inducibility.

The present invention also provides isolated polynucleotides encoding any peptides

of the present disclosure~~invention~~. These polynucleotides can be used for inducing or preparing APCs with CTL inducibility or can be administered to a subject for inducing immune responses against cancers much like the present peptides.

[0012] 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 object of the present disclosure~~invention~~ is to provide agents or compositions that induce CTLs, such agents or compositions including one or more peptides of the present disclosure~~invention~~ or polynucleotides encoding such peptides. The present disclosure~~invention~~ further contemplates pharmaceutical agents or compositions including one or more peptides of the present invention or polynucleotides encoding such peptides, such agents or compositions formulated for the treatment and/or prophylaxis of cancers, as well as the prevention of postoperative recurrence thereof, such cancers including, but not limited, lung cancer, 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. The present pharmaceutical agents or compositions can include APCs or exosomes that present any of the present peptides instead of/in addition to the present peptides or polynucleotides as active ingredients.

[0013] The peptides and polynucleotides of the present disclosure~~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 a polynucleotide encoding a peptide of the present disclosure~~invention~~ into APCs. Such APCs have high CTL inducibility against target peptides and thus find use in cancer immunotherapy. Accordingly, the present disclosure~~invention~~ contemplates both methods for inducing APCs with CTL inducibility and APCs obtained by such methods.

[0014] The present disclosure~~invention~~ also provides methods for inducing CTL, methods that include the step of co-culturing CD8 positive cells with APCs or exosomes presenting one or more peptides of the present disclosure~~invention~~ on its surface or the step of introducing a gene that includes a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide capable of bind to the present peptide. CTLs obtained by such methods find use in the treatment and prevention of cancers, examples of which include, but are not limited to, lung cancer, 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. Therefore, the present disclosure~~invention~~ encompasses the CTLs obtained by the present methods.

It is another object of the present disclosure~~invention~~ to provide methods for inducing an immune response against cancers in a subject in need thereof, such methods including the step of administering to the subject a agent or composition that includes a TTK polypeptide or an immunologically active fragment thereof, polynucleotides encoding a TTK polypeptide, and exosomes or APCs presenting a TTK polypeptide.

[0015] The applicability of the present disclosure~~invention~~ extends to any of a number of diseases relating to or arising from TTK overexpression, such as cancer, exemplary cancers including, but not limited to, lung cancer, 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.

[0015a] In a first aspect, there is provided an isolated peptide, having cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide consists of an amino acid sequence of SEQ ID NO: 3, or consists of an amino acid sequence of SEQ ID NO:3 in which 1 or 2 amino acid(s) are substituted or added.

[0015b] In a second aspect, there is provided a composition for inducing CTL, wherein the agent comprises one or more of the peptide(s) as defined above.

[0015c] In a third aspect, there is provided a pharmaceutical composition for the treatment of cancer, wherein the composition comprises one or more of the peptide(s) as defined above.

[0015d] In a fourth aspect, there is provided a method for inducing an antigen-presenting cell (APC) with CTL inducibility, comprising contacting an APC with the peptide as defined above in vitro or ex vivo.

[0015e] In a fifth aspect, there is provided a diagnostic kit comprising the peptide as defined above.

[0015f] In a sixth aspect, there is provided the pharmaceutical composition as defined above, wherein the composition is formulated for the treatment of cancer.

[0015g] In a seventh aspect, there is provided a method for inducing CTL, comprising a step selected from the group consisting of: (a) co-culturing CD8 positive T cells with APCs that presents on its surface a complex of an HLA antigen and the peptide as defined above; and (b) co-culturing CD8 positive T cells with exosomes that presents on its surface a complex of an HLA antigen and the peptide as defined above.

[0016] Specifically, the present disclosure~~invention~~ provides the following [1] to [21];

[1] An isolated peptide comprising an amino acid sequence of SEQ ID NO: 3.

[2] An isolated peptide, having cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide comprises an amino acid sequence of SEQ ID NO: 3, wherein 1, 2, or several amino acid(s) are substituted, deleted, or added,

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

(a) second amino acid from the N-terminus is or is modified to be an amino acid selected from the group consisting of leucine and methionine; and

(b) C-terminal amino acid is or is modified to be an amino acid selected from the group consisting of valine and leucine,

[4] The isolated peptide of any one of [1] to [3], wherein said peptide is nonapeptide,

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

[6]An agent for inducing CTL, wherein the agent comprises one or more of the peptide(s) of any one of [1] to [4], or one or more of the polynucleotide(s) of [5],

[7]A pharmaceutical agent for the treatment and/or prophylaxis of cancer, and/or the prevention of a postoperative recurrence thereof, wherein the agent comprises one or more of the peptide(s) of any one of [1] to [4], or one or more of the polynucleotides of [5],

[8] The pharmaceutical agent of [7] formulated for the administration to a subject whose HLA-A antigen is HLA-A2,

[9] The pharmaceutical agent of [8], wherein the HLA-A2 is HLA-A*0201,

[10] The pharmaceutical agent of [7] or [8], wherein the agent is formulated for the treatment of cancer,

[11] A method for inducing an antigen-presenting cell (APC) with CTL inducibility, comprising a step selected from the group consisting of:

CLAIMS

~~[Claim 1]~~
~~An isolated peptide comprising an amino acid sequence of SEQ ID NO: 3.~~
[Claim ~~2~~1]
An isolated peptide, having cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide co~~mprises~~nsists of an amino acid sequence of SEQ ID NO: 3, or consists of an amino acid sequence of SEQ ID NO:3 in which~~wherein~~ 1 or~~,~~ 2~~, or several~~ amino acid(s) are substituted~~, deleted,~~ or added.

[Claim ~~2~~3]
The isolated peptide of claim ~~2~~1, wherein the peptide has one or both of the following characteristics:
(a) a second amino acid from the N-terminus of the amino acid sequence of SEQ ID NO:3 ~~is or~~ is modified to be an amino acid selected from the group consisting of leucine and methionine; and
(b) a C-terminal amino acid of the amino acid sequence of SEQ ID NO.3 is ~~or is~~ modified to be an amino acid selected from the group consisting of ~~valine and~~ leucine.

[Claim ~~3~~4]
The isolated peptide of ~~any one of~~ claim~~s~~ 1 ~~to 3~~, wherein said peptide is nonapeptide.
~~[Claim 5]~~
~~An isolated polynucleotide encoding the peptide of any one of claims 1 to 4.~~

[Claim ~~6~~4]
A compositio~~n agent~~ for inducing CTL, wherein the agent comprises one or more of the peptide(s) of ~~any one of~~ claim~~s~~ 1~~, to 4, or one or more of the polynucleotide(s) of claim 5.~~

[Claim ~~5~~7]
A pharmaceutical compositio~~nagent~~ for the treatment ~~and/or prophylaxis~~ of cancer~~, and/or the prevention of a postoperative recurrence thereof,~~ wherein the ~~agent~~ composition comprises one or more of the peptide(s) of ~~any one of~~ claim~~s~~ 1 ~~to 4, or one or more of the polynucleotides of claim 5~~.

[Claim ~~6~~8]
The pharmaceutical compositio~~nagent~~ of claim ~~7~~5, wherein the composition is formulated for the administration to a subject whose HLA-A antigen is HLA-A2.

[Claim ~~7~~9]
The pharmaceutical compositio~~nagent~~ of claim ~~6~~8, wherein the HLA-A2 is HLA-A*0201.

[Claim 8~~10~~]
The pharmaceutical compositionagent of claim 5~~7 or 8~~, wherein the compositionagent is formulated for the treatment of cancer.

[Claim 9~~11~~]
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 the peptide of ~~any one of~~ claim~~s~~ 1 ~~to 4~~ in vitro or~~,~~ ex vivo ~~or in vivo, and~~
~~(b) introducing a polynucleotide encoding the peptide of any one of claims 1 to 4 into an APC.~~

[Claim 10]

A diagnostic kit comprising the peptide of claim 1.

[Claim 11]

The pharmaceutical composition of claim 6, wherein the composition is formulated for the treatment of cancer.

[Claim 12~~2~~]
A method for inducing CTL, comprising a step selected from the group consisting of:
(a) co-culturing CD8 positive T cells with APCs that presents on its surface a complex of an HLA antigen and the peptide of ~~any one of~~ claim~~s~~ 1 ~~to 4~~; and
(b) co-culturing CD8 positive T cells with exosomes that presents on its surface a complex of an HLA antigen and the~~a~~ peptide ~~as of any one~~ of claim~~s~~ 1~~, to 4; and~~
~~(c) introducing a gene that comprises a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide capable of binding to the peptide of any one of claims 1 to 4 into a T cell.~~

~~[Claim 13]~~
~~An isolated APC that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 4.~~

~~[Claim 14]~~
~~The APC of claim 13, which is induced by the method of claim 11.~~

~~[Claim 15]~~
~~An isolated CTL that targets the peptide of any one of claims 1 to 4.~~

~~[Claim 16]~~
~~The CTL of claim 15, which is induced by the method of claim 12.~~

[Claim 13~~7~~]
Use of an agent comprising one or more peptide(s) of claim 1 in the manufacture of a medicament ~~A method of~~for inducing an immune response against cancer in a subject~~, wherein the method comprises administering to the subject an agent comprising one or more peptide(s) of any one of claims 1 to 4, one or more immunologically active fragment(s) thereof, or one or more polynucleotide(s) encoding the peptide(s) or the fragment(s)~~.

~~[Claim 18]~~
~~An antibody or fragment thereof against the peptides of any one of claims 1 to 4.~~

[Claim 19]
A vector comprising a nucleotide sequence encoding the peptide of any one of claims 1 to 4.

[Claim 20]
A host cell transformed or transfected with the vector of claim 19.

[Claim 21]
A diagnostic kit comprising the peptide of any one of claims 1 to 4, the nucleotide of claim 5 or the antibody of claim 18.