binding the mature portion of unfolded proteins and aiding their import into mitochondria (NPL 15).

In a recent study, it was indicated that TOMM34 is frequently up-regulated in colorectal cancer by gene-expression profile analysis using cDNA microarray consisting of 23040 genes. Furthermore, knockdown of TOMM34 by siRNA in colon cancer cell lines attenuated the growth of colon cancer cells (NPL 16).

Citation List

Patent Literature

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Summary of Invention

[0007] In one aspect, there is provided an isolated peptide of less than 15 amino acids, wherein the peptide is (a) or (b): (a) an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 5, 31 and 32; (b) an isolated peptide comprising an amino acid sequence in which one or two amino acid(s) are substituted, inserted or added to an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 5, 31 and 32 to yield a modified peptide that retains cytotoxic T lymphocyte (CTL) inducibility. In another aspect, there is provided a composition for inducing CTL, wherein the composition comprises one or more peptide(s) of the present invention. In another aspect, there is provided a pharmaceutical composition comprising: (a) one or more peptide(s) of the present invention; (b) one or more APCs or exosome(s) that present a complex of the peptide of the present invention and an HLA antigen on their surface; or (c) one or more CTL(s) that recognize a cell

presenting a complex of the peptide of the present invention and an HLA antigen on its surface; in combination with pharmaceutically acceptable carrier, formulated for a purpose selected from the group consisting of: (i) treatment of an existing cancer, (ii) prophylaxis of a cancer, (iii) prevention of a postoperative recurrence of a cancer, and (vi) combinations thereof. In another aspect, there is provided 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 the present invention in vitro; and (b) introducing a polynucleotide encoding the peptide of the present invention into an APC. In another aspect, there is provided an in vitro method for inducing a CTL, comprising a step selected from the group consisting of: (a) co-culturing a CD8-positive T cell with an APC that presents on its surface a complex of an HLA antigen and the peptide of the present invention; (b) co-culturing a CD8-positive T cell with an exosome that presents on its surface a complex of an HLA antigen and the peptide of the present invention, and (c) introducing into a T cell a polynucleotide/polynucleotides encoding T cell receptor (TCR) subunit polypeptides, wherein the TCR formed by said TCR subunit polypeptides is capable of binding to a complex of an HLA antigen and the peptide of the present invention on a cell surface. In another aspect, there is provided an isolated APC that presents on its surface a complex of an HLA antigen and the peptide of the present invention. In another aspect, there is provided an isolated CTL that targets the peptides of the present invention. In another aspect, there is provided use of the peptide of the present invention, or a polynucleotide encoding the peptide, in the manufacture of a medicament for inducing an immune response against cancer in a subject. In another aspect, there is provided a vector comprising a nucleotide sequence encoding the peptide of the present invention. In another aspect, there is provided a host cell transformed or transfected with an expression vector of the present invention. In another aspect, there is provided an exosome that presents a complex comprising the peptide of the present invention and an HLA antigen. In another aspect, there is provided an isolated antibody against the peptide of the present invention, or immunologically active fragment thereof.

The present disclosure is based, at least in part, on the discovery of novel peptides that may serve as suitable targets of immunotherapy. Because TAAs are generally perceived by the immune system as "self" and therefore often have no immunogenicity, the discovery of appropriate targets is of extreme importance. As noted above, TOMM34 (for example, SEQ ID NOs: 41 and 42, also indicated in GenBank Accession No. NM_006809) has been identified as up-regulated in cancers, including, but are not limited to, acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), bladder cancer, breast cancer, cervical cancer, colorectal cancer, esophagus cancer, liver cancer, osteosarcoma, prostate cancer, renal carcinoma, small

cell lung cancer (SCLC), non-small cell lung cancer (NSCLC) and soft tissue tumor. Thus, the present disclosure focuses on TOMM34 as an appropriate cancer marker and a candidate for the target of immunotherapy.

[0008] In the course of the present disclosure, specific epitope peptides of the gene products of TOMM34 that possess the ability to induce CTLs specific to TOMM34 were identified. As discussed in greater detail below, peripheral blood mononuclear cells (PBMCs) obtained from a healthy donor were stimulated using HLA-A*0201 binding candidate peptides derived from TOMM34. CTL lines were then established with specific cytotoxicity against the HLA-A2 positive target cells pulsed with each of candidate peptides. The results herein demonstrate that these peptides are HLA-A2 restricted epitope peptides that may induce potent and specific immune responses against cells expressing TOMM34. These results further indicate that TOMM34 is strongly immunogenic and the epitopes thereof are effective targets for tumor immunotherapy.

[0009] Accordingly, it is an object of the present disclosure to provide isolated peptides that bind to HLA antigen and include the amino acid sequence of TOMM34 (SEQ ID NO: 42) or the immunologically active fragments thereof. These peptides are expected to have CTL inducibility and, thus, can be used to induce CTL in vitro or ex vivo, or to be administered to a subject for inducing immune responses against cancers, examples of which include, but are not limited to AML, CML, bladder cancer, breast cancer, cervical cancer, colorectal cancer, esophagus cancer, liver cancer, osteosarcoma, prostate cancer, renal carcinoma, SCLC, NSCLC and soft tissue tumor. In some examples, the peptides are nonapeptides or decapeptides. In some examples, the peptide is a nonapeptide or decapeptide consisting of an amino acid sequence selected from among SEQ ID NOs: 1 to 20 and 22 to 40. In some examples, the peptides having an amino acid sequence selected from among SEQ ID NOs: 1, 5, 31 and 32 showed particularly strong CTL inducibility.

[0010] The present disclosure also contemplates modified peptides having an amino acid sequence of an immunologically active fragment of TOMM34 in which one, two or more amino acids are substituted, deleted, inserted or added, so long as the modified peptides retain the requisite CTL inducibility of the original unmodified peptide. In some examples, the peptide has an amino sequence of SEQ ID NO: 1, 5, 31 or 32 in which one, two or more amino acids are substituted, deleted, inserted or added.

The present disclosure further encompasses isolated polynucleotides encoding any peptides of the present disclosure. These polynucleotides can be used to induce or prepare APCs having CTL inducibility. Like the above-described peptides of the present disclosure, such APCs can be administered to a subject for inducing immune responses against cancers.

[0011] When administered to a subject, the present peptides are presented on the surface of APCs so as to induce CTLs targeting the respective peptides. Therefore, one object of the present disclosure is to provide compositions or agents including any peptides or polynucleotides provided by the present disclosure for inducing CTL. Such compositions or agents, including any peptides or polynucleotides, can be used for the treatment and/or prophylaxis of cancers or the prevention of a postoperative recurrence of cancer, examples of which include, but are not limited to, AML, CML, bladder cancer, breast cancer, cervical cancer, colorectal cancer, esophagus cancer, liver cancer, osteosarcoma, prostate cancer, renal carcinoma, SCLC, NSCLC and soft tissue tumor, and/or preventing postoperative recurrence thereof.

[0012] The present disclosure also contemplates pharmaceutical agents or compositions that include or incorporate one or more peptides or polynucleotides of the present disclosure formulated for the treatment and/or prophylaxis of cancer, particularly a primary cancer, or the prevention of a postoperative recurrence thereof. Instead of or in addition to the present peptides or polynucleotides, the present pharmaceutical agents or compositions may include as active ingredients APCs or exosomes that present any of the present peptides.

[0013] The peptides or polynucleotides of the present disclosure may be used to induce APCs which 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 this disclosure into APCs. Such APCs have high CTL inducibility against target peptides and are useful for cancer immunotherapy. Accordingly, the present disclosure contemplates both methods for inducing APCs with CTL inducibility and the APCs obtained by such methods.

[0014] It is a further object of the present disclosure to provide methods for inducing CTLs, such methods including the step of co-culturing CD8-positive cells with APCs or exosomes presenting the peptide of the present disclosure on its surface or the step introducing a polynucleotide/polynucleotides encoding T cell receptor (TCR) subunit polypeptides, wherein the TCR formed by such subunit polypeptides is capable of binding to a complex of an HLA antigen and the present peptide on a cell surface. CTLs obtained by such methods are useful in the treatment and prevention of cancers, examples of which include, but are not limited to, AML, CML, bladder cancer, breast cancer, cervical cancer, colorectal cancer, esophagus cancer, liver cancer, osteosarcoma, prostate cancer, renal carcinoma, SCLC, NSCLC and soft tissue tumor.

Yet another object of the present disclosure is to provide isolated APCs that present on the surface a complex of an HLA antigen and a peptide of the present disclosure. The present disclosure further provides isolated CTLs that target peptides of the present disclosure. These APCs and CTLs may be used for cancer immunotherapy.

[0015] It is yet another object of the present disclosure to provide methods for inducing an immune response against a cancer in a subject in need thereof, such methods including the step of administering to the subject a composition or agent that include one or more peptides of the present disclosure, polynucleotides encoding the peptide of the present disclosure, or exosomes or APCs presenting the peptide of the present disclosure.
The applicability of the present disclosure extends to any of a number of diseases relating to or arising from TOMM34 overexpression, such as cancer, exemplary cancers including, but not limited to, AML, CML, bladder cancer, breast cancer, cervical cancer, colorectal cancer, esophagus cancer, liver cancer, osteosarcoma, prostate cancer, renal carcinoma, SCLC, NSCLC and soft tissue tumor.

[0016] More specifically, the present disclosure provides the following [1] to[20]:

[1]An isolated peptide following (a) or (b):

(a) an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 5 31 and 32;

(b) an isolated peptide comprising an amino acid sequence in which one, two, or several amino acid(s) are substituted, deleted, inserted or added to an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 5, 31 and 32 to yield a modified peptide that retains the ability to bind to an HLA antigen and cytotoxic T lymphocyte (CTL) inducibility;

[2]The isolated peptide of [1], wherein the HLA antigen is HLA-A2;

[3]The isolated peptide of [1] or [2], wherein said peptide is a nonapeptide or decapeptide;

[4]The peptide of any one of [1] to [3], having at least one substitution selected from the group consisting of:

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

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

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

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

[7]A pharmaceutical composition comprising:

(a) one or more peptide(s) of any one of [1] to [4];

(b) one or more polynucleotides of [5];

(c) one or more APCs or exosomes that present a complex of the peptide of any one of [1] to [4] and an HLA antigen on their surface; or

(d) one or more CTLs that recognize a cell presenting a complex of the peptide of any one of [1] to [4] and an HLA antigen on its surface,

Claims

[Claim 1] An isolated peptide of less than 15 amino acids, wherein the peptide is (a) or (b):
(a) an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 5, 31 and 32;
(b) an isolated peptide comprising an amino acid sequence in which one or two amino acid(s) are substituted, inserted or added to an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 5, 31 and 32 to yield a modified peptide that retains cytotoxic T lymphocyte (CTL) inducibility.

[Claim 2] The isolated peptide of claim 1, wherein the peptide consists of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 5, 31 and 32.

[Claim 3] The peptide of claim 1 or 2, having at least one substitution selected from the group consisting of:
(a) the second amino acid from N-terminus of the amino acid sequence of SEQ ID NO: 1, 5, 31 or 32 is substituted with leucine or methionine; and
(b) the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 1, 5, 31 or 32 is substituted with valine or leucine.

[Claim 4] A composition for inducing CTL, wherein the composition comprises one or more peptide(s) of any one of claims 1 to 3.

[Claim 5] A pharmaceutical composition comprising:
(a) one or more peptide(s) of any one of claims 1 to 3;
(b) one or more APCs or exosome(s) that present a complex of the peptide of any one of claims 1 to 3 and an HLA antigen on their surface; or
(c) one or more CTL(s) that recognize a cell presenting a complex of the peptide of any one of claims 1 to 3 and an HLA antigen on its surface,
in combination with pharmaceutically acceptable carrier,
formulated for a purpose selected from the group consisting of:
(i) treatment of an existing cancer,
(ii) prophylaxis of a cancer,
(iii) prevention of a postoperative recurrence of a cancer, and
(vi) combinations thereof.

[Claim 6] The pharmaceutical composition of claim 5 formulated for administration to a subject whose HLA antigen is HLA-A2.

[Claim 7] 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 claims 1 to 3 *in vitro*; and
(b) introducing a polynucleotide encoding the peptide of any one of claims 1 to 3 into an APC.

[Claim 8] An *in vitro* method for inducing a CTL, comprising a step selected from the group consisting of:
(a) co-culturing a CD8-positive T cell with an APC that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 3;
(b) co-culturing a CD8-positive T cell with an exosome that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 3, and
(c) introducing into a T cell a polynucleotide/polynucleotides encoding T cell receptor (TCR) subunit polypeptides, wherein the TCR formed by said TCR subunit polypeptides is capable of binding to a complex of an HLA antigen and the peptide of any one of claims 1 to 3 on a cell surface.

[Claim 9] An isolated APC that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 3.

[Claim 10] The APC of claim 9, which is induced by a method for inducing an antigen-presenting cell (APC) with CTL inducibility, wherein the method comprises a step selected from the group consisting of:
(a) contacting an APC with the peptide of any one of claims 1 to 3 *in vitro*; and
(b) introducing a polynucleotide encoding the peptide of any one of claims 1 to 3 into an APC.

[Claim 11] An isolated CTL that targets the peptides of any one of claims 1 to 3.

[Claim 12] The CTL of claims 11, which is induced by an *in vitro* method for inducing a CTL, wherein the *in vitro* method comprises a step selected from the group consisting of:
(a) co-culturing a CD8-positive T cell with an APC that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 3;
(b) co-culturing a CD8-positive T cell with an exosome that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 3; and
(c) introducing into a T cell a polynucleotide/polynucleotides encoding T cell receptor (TCR) subunit polypeptides, wherein the

TCR formed by said TCR subunit polypeptides is capable of binding to a complex of an HLA antigen and the peptide of any one of claims 1 to 3 on a cell surface.

[Claim 13] Use of the peptide of any one of claims 1 to 3, or a polynucleotide encoding the peptide, in the manufacture of a medicament for inducing an immune response against cancer in a subject.

[Claim 14] A vector comprising a nucleotide sequence encoding the peptide of any one of claims 1 to 3.

[Claim 15] A host cell transformed or transfected with an expression vector of claim 14.

[Claim 16] An exosome that presents a complex comprising the peptide of any one of claims 1 to 3 and an HLA antigen.

[Claim 17] An isolated antibody against the peptide of any one of claims 1 to 3, or immunologically active fragment thereof.