UBE2T binds FANCL, and is necessary for the DNA damage-induced monoubiquitination of FANCD2 (NPL 15-16). In recent studies, UBE2T was found to be frequently up-regulated in breast cancers, and interact and co-localize with the BRCA1/ BRCA1-associated RING domain protein (BARD1) complex (PTL 1, NPL 17). Northern blot analysis in those studies revealed that UBE2T transcript was detected at very high level in breast cancer cell lines, but hardly detected in the vital organs. Furthermore, knockdowns of endogenous UBE2T by siRNA in cancer cell lines have been shown to significantly suppress growth of those cell lines (PTL 1-2, NPL 17).

Citation List

Patent Literature

[0006] [PTL 1] WO2005/029067

[PTL 2] WO2009/001562

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Summary of Invention

[0008] 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 innate immunogenicity, the discovery of appropriate targets is still of importance.

To that end, the present disclosure is directed, at least in part, to the identification of

specific epitope peptides that possess the ability to induce CTLs specific to UBE2T among peptides derived from UBE2T.

[0009]

The results disclosed herein demonstrate that identified peptides are HLA-A24 or HLA-A2 restricted epitope peptides that can induce potent and specific immune responses against cells expressing UBE2T.

[0010]

Accordingly, it is an object of the present disclosure to provide UBE2T-derived peptides that can be used to induce CTLs in vitro, ex vivo or in vivo, or to be administered to a subject for inducing immune responses against cancers, examples of which include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophageal cancer, gastric cancer, diffuse-type gastric cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, SCLC, soft tissue tumor and testicular tumor. In one aspect, the present disclosure refers to an isolated peptide of less than 15 amino acids capable of inducing cytotoxic T lymphocytes (CTLs), wherein the peptide comprises an amino acid sequence (a) or (b) bellow:

(a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 4, 6, 11, 12, 13, 15, 17, 19, 20, 21, 22, 23, 24, 25, 27, 29, 30, 32, 36, 38, 41, 48, 49, 51, 52, 53, 55, 56 and 58; or

(b) an amino acid sequence in which 1 or 2 amino acid(s) are substituted and/or added in the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 4, 6, 11, 12, 13, 15, 17, 19, 20, 21, 22, 23, 24, 25, 27, 29, 30, 32, 36, 38, 41, 48, 49, 51, 52, 53, 55, 56 and 58;

wherein the substitutions are selected from the group consisting of (i) and (ii) as follows:

(i) a peptide that has one or both of the following characteristics:

(a) the second amino acid from the N-terminus of the amino acid sequence of SEQ ID NO: 1, 2, 4, 6, 11, 12, 13, 15, 17, 19, 20, 21, 22, 23, 24, 25 or 27 is substituted with phenylalanine, tyrosine, methionine or tryptophan; and

(b) the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 1, 2, 4, 6, 11, 12, 13, 15, 17, 19, 20, 21, 22, 23, 24, 25 or 27 is substituted with phenylalanine, leucine, isoleucine, tryptophan or methionine; or

(ii) a peptide that has one or both of the following characteristics:

(a) the second amino acid from the N-terminus of the amino acid sequence of SEQ ID NO: 48, 29, 30, 32, 36, 38, 41, 49, 51, 52, 53, 55, 56 or 58 is substituted with leucine or methionine; and

(b) the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 48, 29, 30, 32, 36, 38, 41, 49, 51, 52, 53, 55, 56 or 58 is substituted with valine or leucine; and
wherein the amino acids, if added, are added to the N and/or C-terminus of the peptide. Preferred peptides are nonapeptides and decapeptides, more preferably nonapeptides and decapeptides having an amino acid sequence selected from among SEQ ID NOs: 1, 2, 4, 6, 11, 12, 13, 15, 17, 19, 20, 21, 22, 23, 24, 25, 27, 29, 30, 32, 36, 38, 41, 48, 49, 51, 52, 53, 55, 56 and 58. Of these, the peptides having an amino acid sequence selected from among SEQ ID NOs: 1, 2, 4, 6, 11, 12, 13, 15, 17, 19, 20, 21, 22, 23, 24, 25, 27, 29, 30, 32, 36, 38, 41, 48, 49, 51, 52, 53, 55, 56 and 58 are particularly preferred.

[0011]

The present disclosure also contemplates modified peptides having an amino acid sequence selected from among SEQ ID NOs: 1, 2, 4, 6, 11, 12, 13, 15, 17, 19, 20, 21, 22, 23, 24, 25, 27, 29, 30, 32, 36, 38, 41, 48, 49, 51, 52, 53, 55, 56 and 58 in which one, two or more amino acids are substituted, deleted, inserted and/or added, as long as the resulting modified peptides retain the requisite CTL inducibility of the original unmodified peptide. In one example, when the original peptides is 9mer (SEQ ID NOs: 1, 2, 4, 6, 11, 12, 13, 15, 29, 30, 32, 36, 38, and 41), the size of the modified peptide is preferably in the range of 9 to 40 amino acids, such as in the range of 9 to 20 amino acids, for example in the range 9 to 15 amino acids. Likewise, when the original peptides is 10mer (SEQ ID NOs: 17, 19, 20, 21, 22, 23, 24, 25, 27, 48, 49, 51, 52, 53, 55, 56 and 58), the size of the modified peptide is preferably in the range of 10 to 40 amino acids, such as in the range of 10 to 20 amino acids, for example in the range 10 to 15 amino acids.

[0012]

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

[0013]

When administered to a subject, the peptides of the present disclosure can be

presented on the surface of APCs so as to induce CTLs targeting the respective peptides. In another aspect, the present disclosure refers to a composition for inducing a CTL, wherein the composition comprises at least one active ingredient selected from the group consisting of: (a) the peptide as disclosed herein; (b) the polynucleotide as disclosed herein; (c) an antigen-presenting cell (APC) that presents the peptide as disclosed herein on its surface; and (d) an exosome that presents the peptide as disclosed herein on its surface. Therefore, one object of the present disclosure is to provide agents or compositions including one or more peptides of the present disclosure, or polynucleotides encoding such peptides. The agent or composition may be used for inducing a CTL. Such agents or compositions can be used for the treatment and/or prophylaxis of a cancer, and/or the prevention of a metastasis or post-operative recurrence thereof. Examples of cancers contemplated by the present disclosure include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophageal cancer, gastric cancer, diffuse-type gastric cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, SCLC, soft tissue tumor and testicular tumor.

[0014]

In another aspect, the present disclosure refers to a pharmaceutical composition for the treatment and/or prophylaxis of cancer, and/or the prevention of a postoperative recurrence thereof, wherein the composition comprises at least one active ingredient selected from the group consisting of: (a) the peptide as disclosed herein; (b) the polynucleotide as disclosed herein; (c) an APC that presents the peptide as disclosed herein on its surface; (d) an exosome that presents the peptide as disclosed herein on its surface; and (e) a CTL that can recognize a cell presenting the peptide as disclosed herein. The present disclosure further contemplates pharmaceutical compositions or agents that include one or more peptides or polynucleotides of the present disclosure. The pharmaceutical composition is preferably formulated for use in the treatment and/or prophylaxis of a cancer, and/or prevention of metastasis or postoperative recurrence thereof. Instead of or in addition to the peptides or polynucleotides of the present disclosure, the pharmaceutical agents or compositions of the present disclosure may include as active ingredients APCs and/or exosomes that present any of the peptides of the present disclosure.

[0015]

In another aspect, the present disclosure refers to an *in vitro* method for inducing an APC capable of inducing CTLs, wherein the method comprises the step selected from the group consisting of: (a) contacting an APC with the peptide as disclosed herein, and

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

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

[0016]

In another aspect, the present disclosure refers to an *in vitro* method for inducing a CTL, wherein the method comprises a step selected from the group consisting of: (a) co-culturing a CD8 positive T cell with an APC that presents on its surface a complex of an HLA antigen and the peptide as disclosed herein; and (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 as disclosed herein. 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 T cells with APCs presenting on its surface a complex of an HLA antigen and the peptide of the present disclosure, the step of co-culturing CD8 positive T cells with exosomes presenting on its surface a complex of an HLA antigen and the peptide of the present disclosure, or the step of introducing a polynucleotide encoding both of T cell receptor (TCR) subunits or polynucleotides encoding each of TCR subunits, wherein the TCR can bind

to a complex of the peptide of the present disclosure and an HLA antigen presented on cell surface. CTLs obtained by such methods can find use in the treatment and/or prevention of cancers, examples of which include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophageal cancer, gastric cancer, diffuse-type gastric cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, SCLC, soft tissue tumor and testicular tumor. Therefore, the present disclosure also encompasses CTLs obtained by above-described methods.

[0017]

In yet another aspect, 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. In yet another aspect, the present disclosure further provides isolated CTLs that target peptides of the present disclosure. Such CTLs may be also defined as CTLs that can recognize (or bind to) a complex of a peptide of the present disclosure and an HLA antigen on the cell surface. These APCs and CTLs may be used for cancer immunotherapy.

[0018]

In yet another aspect, the present disclosure refers to a use of a composition in the manufacture of a medicament for inducing an immune response against cancer in a subject in need thereof, wherein the composition comprises the peptide as disclosed herein, or a polynucleotide encoding the peptide. 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 an agent or composition including at least one component selected from among a peptide of the present disclosure or a polynucleotide encoding thereof, an APC or exosome presenting thereof and a CTL that can recognize a cell presenting the peptide of the present disclosure on the surface.

[0019]

One example of the present disclosure pertains to a peptide of the present disclosure or a composition comprising a peptide of the present disclosure for use a medicament.

The applicability of the present disclosure extends to any of a number of diseases relating to or arising from UBE2T overexpression, such as cancer, examples of which include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophageal cancer, gastric cancer, diffuse-type gastric cancer, NSCLC, lymphoma, osteosarcoma, ovarian cancer,

pancreatic cancer, prostate cancer, SCLC, soft tissue tumor and testicular tumor.

In yet another aspect, the present disclosure refers to a vector comprising a nucleotide sequence encoding the peptide as disclosed herein.

[0020]

More specifically, the present disclosure provides followings:

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

(a) an amino acid sequence of an immunologically active fragment of UBE2T;

(b) an amino acid sequence in which 1, 2, or several amino acid(s) are substituted, deleted, inserted and/or added in an amino acid sequence of an immunologically active fragment of UBE2T,

wherein the CTL induced by the peptide has specific cytotoxic activity against a cell that presents a fragment derived from UBE2T;

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

Claims

[Claim 1]
An isolated peptide of less than 15 amino acids capable of inducing cytotoxic T lymphocytes (CTLs), wherein the peptide comprises an amino acid sequence (a) or (b) bellow:
(a) an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 4, 6, 11, 12, 13, 15, 17, 19, 20, 21, 22, 23, 24, 25, 27, 29, 30, 32, 36, 38, 41, 48, 49, 51, 52, 53, 55, 56 and 58; or
(b) an amino acid sequence in which 1 or 2 amino acid(s) are substituted and/or added in the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 4, 6, 11, 12, 13, 15, 17, 19, 20, 21, 22, 23, 24, 25, 27, 29, 30, 32, 36, 38, 41, 48, 49, 51, 52, 53, 55, 56 and 58;
wherein the substitutions are selected from the group consisting of (i) and (ii) as follows:
(i) a peptide that has one or both of the following characteristics:
(a) the second amino acid from the N-terminus of the amino acid sequence of SEQ ID NO: 1, 2, 4, 6, 11, 12, 13, 15, 17, 19, 20, 21, 22, 23, 24, 25 or 27 is substituted with phenylalanine, tyrosine, methionine or tryptophan; and
(b) the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 1, 2, 4, 6, 11, 12, 13, 15, 17, 19, 20, 21, 22, 23, 24, 25 or 27 is substituted with phenylalanine, leucine, isoleucine, tryptophan or methionine; or
(ii) a peptide that has one or both of the following characteristics:
(a) the second amino acid from the N-terminus of the amino acid sequence of SEQ ID NO: 48, 29, 30, 32, 36, 38, 41, 49, 51, 52, 53, 55, 56 or 58 is substituted with leucine or methionine; and
(b) the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 48, 29, 30, 32, 36, 38, 41, 49, 51, 52, 53, 55, 56 or 58 is substituted with valine or leucine; and
wherein the amino acids, if added, are added to the N and/or C-terminus of the peptide.

[Claim 2]
The peptide of claim 1, wherein the peptide consists of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 4, 6, 11, 12, 13, 15, 17, 19, 20, 21, 22, 23, 24, 25, 27, 29, 30, 32, 36, 38, 41, 48, 49, 51, 52, 53, 55, 56 and 58.

[Claim 3]
An isolated polynucleotide encoding the peptide of claim 1 or 2.

[Claim 4]
A composition for inducing a CTL, wherein the composition comprises at least one active

ingredient selected from the group consisting of:
(a) the peptide of claim 1 or 2;
(b) the polynucleotide of claim 3;
(c) an antigen-presenting cell (APC) that presents the peptide of claim 1 or 2 on its surface; and
(d) an exosome that presents the peptide of claim 1 or 2 on its surface.

[Claim 5]
A pharmaceutical composition for the treatment and/or prophylaxis of cancer, and/or the prevention of a postoperative recurrence thereof, wherein the composition comprises at least one active ingredient selected from the group consisting of:
(a) the peptide of claim 1 or 2;
(b) the polynucleotide of claim 3;
(c) an APC that presents the peptide of claim 1 or 2 on its surface;
(d) an exosome that presents the peptide of claim 1 or 2 on its surface; and
(e) a CTL that can recognize a cell presenting the peptide of claim 1 or 2.

[Claim 6]
The pharmaceutical composition of claim 5, wherein the pharmaceutical composition is formulated for the administration to a subject whose HLA antigen is HLA-A24 or HLA-A2.

[Claim 7]
An *in vitro* method for inducing an APC capable of inducing CTLs, wherein the method comprises the step selected from the group consisting of:
(a) contacting an APC with the peptide of claim 1 or 2, and
(b) introducing a polynucleotide encoding the peptide of claim 1 or 2 into an APC.

[Claim 8]
An *in vitro* method for inducing a CTL, wherein the method comprises a step selected from the group consisting of:
(a) co-culturing a CD8 positive T cell with an APC that presents on its surface a complex of an HLA antigen and the peptide of claim 1 or 2; and
(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 claim 1 or 2.

[Claim 9]
An isolated APC that presents on its surface a complex of an HLA antigen and the peptide of claim 1 or 2.

[Claim 10]
An isolated CTL that targets the peptide of claim 1 or 2.

[Claim 11]
Use of a composition in the manufacture of a medicament for inducing an immune response against cancer in a subject in need thereof, wherein the composition comprises the peptide of claim 1 or 2, or a polynucleotide encoding the peptide.

[Claim 12]
A vector comprising a nucleotide sequence encoding the peptide of claim 1 or 2.