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Summary

[0009] The present disclosure is based in part on the discovery of a new application of two peptides, which have an amino acid sequence shown in SEQ ID NO: 1 or SEQ ID NO: 2. In the context of the present disclosure, peripheral blood mononuclear cells (PBMCs) obtained from a healthy donor were stimulated with candidate peptides derived from HIG2 or URLC10. CTLs that specifically recognize HLA-A0206 positive target cells pulsed with the respective candidate peptides were established, and HLA-A0206 restricted epitope peptides that can induce potent and specific immune responses against HIG2 or URLC10 presented on the surface of target cells were identified.

[0010] Accordingly, it is an object of the present disclosure to provide peptides having CTL inducibility as well as an amino acid sequence of SEQ ID NOs: 1 or 2. In addition, the present disclosure contemplates the use of modified peptides, wherein one, two or more amino acids are substituted, deleted, inserted and/or added, so long as the resulting modified peptides retain the CTL inducibility of the original peptide.

[0011] When administered to a subject whose HLA antigen is HLA-A0206, the present peptides are presented on the surface of antigen-presenting cells and then induce CTLs targeting the respective peptides. Therefore, it is an object of the present disclosure to provide antigen-presenting cells and exosomes that present any of the present peptides with HLA-A0206 antigen, as well as methods for inducing antigen-presenting cells.

[0011a] In one aspect, there is provided an *in vitro* method for inducing an antigen-presenting cell with CTL inducibility, wherein the method comprises the step selected from the group consisting of:

(a) contacting antigen-presenting cells that have been obtained from a subject whose HLA-A antigen is HLA-A0206 with a peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2; and

(b) introducing a polynucleotide into antigen-presenting cells that have been obtained from a subject whose HLA-A antigen is HLA-A0206, wherein the polynucleotide encodes a peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

[0011b] In another aspect, there is provided an *in vitro* method for inducing a CTL which recognizes a complex of an HLA-A0206 antigen and a peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2, wherein the method comprises the steps of;

(a) contacting antigen-presenting cells (APCs) that have been collected from a subject whose HLA-A antigen is HLA-A0206, with a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2;

(b) mixing the antigen-presenting cells of step (a) with CD8+ T cells whose HLA-A antigen is HLA-A0206, and co-culturing for inducing CTLs; and

(c) collecting CD8+ T cells from the co-culture of step (b).

[0011c] In yet another aspect, there is provided an isolated antigen-presenting cell, which presents on its surface a complex of an HLA-A0206 antigen and a peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

[0011d] In a further aspect, there is provided an isolated CTL, which recognizes a complex of an HLA-A0206 antigen and a peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

[0012] An anti-tumor immune response is induced by the administration of the present HIG2 or URLC10 polypeptides or polynucleotide encoding the polypeptides, as well as exosomes and antigen-presenting cells which present the HIG2 or URLC10 polypeptides. Therefore, it is yet another object of the present disclosure to provide pharmaceutical agents containing the polypeptides or polynucleotides encoding them, as well as the exosomes and antigen-presenting cells as their active ingredients which are intended for the administration to a subject whose HLA antigen is HLA-A0206. The pharmaceutical agents of the present disclosure find use as vaccines.

[0013] Moreover, it is a further object of the present disclosure to provide methods for the treatment and/or prophylaxis of (i.e., prevention) cancers (tumors), and/or prevention of postoperative recurrence thereof, as well as methods for inducing CTLs, methods for inducing an immune response against cancers (tumors) and also anti-tumor immunity, wherein a subject has HLA-A0206 antigen, such methods including the step of administering the peptides of SEQ ID NO: 1 or SEQ ID NO: 2, exosomes or the antigen-presenting cells presenting SEQ ID NO: 1 or SEQ ID NO: 2 or the pharmaceutical agents of the disclosure.

[0013a] In one aspect, there is provided a use of an active ingredient for manufacturing an agent for inducing an immune response against cancer in a subject whose HLA-A antigen is HLA-A0206, wherein the active ingredient is selected from the group consisting of (a)-(e) as follows:

(a) one or more peptide(s) having CTL inducibility, wherein the peptide(s) consist(s) of an amino acid sequence selected from the group consisting of:

(i) SEQ ID NO: 1; and

(ii) SEQ ID NO: 2;

(b) one or more polynucleotide(s) encoding any one of the peptide(s) of (a);

(c) one or more isolated antigen-presenting cell(s) as disclosed herein;

(d) one or more isolated CTL(s) as disclosed herein; and,

(e) the combination of the active ingredients selected from the group consisting of (a)-(d).

[0013b] In another aspect, there is provided use of an active ingredient for the manufacture of a medicament for a use selected from the group consisting of:

(i) treatment of cancer in a subject whose HLA-A antigen is HLA-A0206,

(ii) prophylaxis of cancer in a subject whose HLA-A antigen is HLA-A0206,

(iii) preventing postoperative recurrence of cancer in a subject whose HLA-A antigen is HLA-A0206, and

(iv) combinations thereof,

wherein the active ingredient is the following (a) or (b):

(a) one or more peptide(s) having cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide consists of an amino acid sequence selected from the group consisting of:

(A) SEQ ID NO: 1; and

(B) SEQ ID NO: 2; or

(b) one or more polynucleotide(s) encoding any one of the peptide(s) of (a).

[0013c] In addition, the CTLs of the present disclosure also find use as vaccines against cancer. Examples of target cancers include, but are not

limited to, renal cancer, bladder cancer, cervical cancer, cholangiocellular carcinoma, esophageal cancer, gastric cancer, NSCLC, osteosarcoma, pancreatic cancer and soft tissue tumor.

[0014] It is to be understood that both the foregoing summary of the disclosure and the following detailed description are of exemplified embodiments, and not restrictive of the disclosure or other alternate embodiments of the disclosure.

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 detailed description of the present invention and its preferred embodiments which follows:

[0016] [fig.1] Figure 1 includes a series of photographs depicting the results of IFN-gamma ELISPOT assay on CTLs that were induced with a peptide derived from HIG2. The CTLs in well #1, #2, #5, #7, #8, #10, #13 and #14 stimulated with HIG2-A0206-9-4 (SEQ ID NO: 1) showed potent IFN-gamma production as compared with the control. In the figures, "+" indicates that the target cells were pulsed with the appropriate peptide, and "-" indicates that the target cells were not been pulsed with any peptides.

[0017] [fig. 2] Figure 2 depicts a series of line graphs showing the results of establishment of CTL lines stimulated with HIG2-A0206-9-4 (SEQ ID NO: 1) with IFN-gamma ELISA assay. It demonstrated that CTL lines established by stimulation with HIG2-A0206-9-4 (SEQ ID NO: 1) showed potent IFN-gamma production as compared with the control. In the figures, "+" indicates that the target cells were pulsed with the appropriate peptide, and "-" indicates that the target cells had not been pulsed with any peptides.

[0018] [fig. 3] Figure 3 depicts a line graph showing the results of the establishment of a CTL clone stimulated with HIG2-A0206-9-4 (SEQ ID NO: 1) with IFN-gamma ELISA assay. The results demonstrate that the CTL clone established by stimulation with HIG2-A0206-9-4 (SEQ ID NO: 1) showed potent IFN-gamma production as compared with the control. In the figures, "+" indicates that the target cells were pulsed with the appropriate peptide, and "-" indicates that the target cells had not been pulsed with any peptides.

[0019] [fig. 4] Figure 4 depicts a line graph showing the specific CTL activity against the target cells that express HIG2 and HLA-A*0206. COS7 cells transfected with HLA-A*0206 alone and pulsed with an inappropriate peptide derived from HIG2, or with HIG2 alone were prepared as control. The CTL clone established with HIG2-A0206-9-4 (SEQ ID NO: 1) showed high specific CTL activity against COS7 cells transfected with both HIG2 and HLA-A0206 (black lozenge-mark). On the other hand, no significant specific CTL activity was detected against target cells expressing either HLA-A0206

CLAIMS

1. An *in vitro* method for inducing an antigen-presenting cell with CTL inducibility, wherein the method comprises the step selected from the group consisting of:
   (a) contacting antigen-presenting cells that have been obtained from a subject whose HLA-A antigen is HLA-A0206 with a peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2; and
   (b) introducing a polynucleotide into antigen-presenting cells that have been obtained from a subject whose HLA-A antigen is HLA-A0206, wherein the polynucleotide encodes a peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

2. An *in vitro* method for inducing a CTL which recognizes a complex of an HLA-A0206 antigen and a peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2, wherein the method comprises the steps of;
   (a) contacting antigen-presenting cells (APCs) that have been collected from a subject whose HLA-A antigen is HLA-A0206, with a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2;
   (b) mixing the antigen-presenting cells of step (a) with CD8+ T cells whose HLA-A antigen is HLA-A0206, and co-culturing for inducing CTLs; and
   (c) collecting CD8+ T cells from the co-culture of step (b).

3. An isolated antigen-presenting cell, which presents on its surface a complex of an HLA-A0206 antigen and a peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

4. An isolated CTL, which recognizes a complex of an HLA-A0206 antigen and a peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and SEQ ID NO: 2.

5. A use of an active ingredient for manufacturing an agent for inducing an

immune response against cancer in a subject whose HLA-A antigen is HLA-A0206, wherein the active ingredient is selected from the group consisting of (a)-(e) as follows:

(a) one or more peptide(s) having CTL inducibility, wherein the peptide(s) consist(s) of an amino acid sequence selected from the group consisting of:
(i) SEQ ID NO: 1; and
(ii) SEQ ID NO: 2;

(b) one or more polynucleotide(s) encoding any one of the peptide(s) of (a);

(c) one or more isolated antigen-presenting cell(s) of claim 3;

(d) one or more isolated CTL(s) of claim 4; and,

(e) the combination of the active ingredients selected from the group consisting of (a)-(d).

6. The use of claim 5, wherein the cancer is bladder cancer, cervical cancer, cholangiocellular carcinoma, esophagus cancer, gastric cancer, non-small cell lung cancer, osteosarcoma, pancreatic cancer, renal carcinoma or soft tissue tumor.

7. Use of an active ingredient for the manufacture of a medicament for a use selected from the group consisting of:

(i) treatment of cancer in a subject whose HLA-A antigen is HLA-A0206,

(ii) prophylaxis of cancer in a subject whose HLA-A antigen is HLA-A0206,

(iii) preventing postoperative recurrence of cancer in a subject whose HLA-A antigen is HLA-A0206, and

(iv) combinations thereof,

wherein the active ingredient is the following (a) or (b):

(a) one or more peptide(s) having cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide consists of an amino acid sequence selected from the group consisting of:
(A) SEQ ID NO: 1; and
(B) SEQ ID NO: 2; or

(b) one or more polynucleotide(s) encoding any one of the peptide(s) of (a).

8. The use of claim 7, wherein said cancer is selected from the group consisting of bladder cancer, cervical cancer, cholangiocellular carcinoma, esophagus cancer, gastric cancer, non-small cell lung cancer (NSCLC), osteosarcoma, pancreatic cancer, renal carcinoma and soft tissue tumor.

9. The use of claim 7, wherein the medicament is formulated as a vaccine.