[NPL 18] Feldman et al., Cell. 1997 Oct 17;91(2):221-30

Summary ~~of Invention~~

[0009] The present ~~invention~~disclosure is based in part on the discovery of 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 of extreme importance. As noted above, recognizing that WDRPUH (SEQ ID NO: 64 encoded by the gene of GenBank Accession No. NM_145054 (SEQ ID NO: 63)) has been identified as up-regulated in cancer tissue of hepatocellular carcinoma, WDRPUH is a candidate target for immunotherapy.

[0010] The present ~~invention~~disclosure is based, at least in part, on the identification of specific epitope peptides of the gene products of WDRPUH which possess the ability to induce CTLs specific to WDRPUH. As discussed in detail below, peripheral blood mononuclear cells (PBMCs) obtained from a healthy donor were stimulated using HLA-A*2402 or HLA-A*0201 binding candidate peptides derived from WDRPUH. CTL lines with specific cytotoxicity against HLA-A24 or HLA-A2 positive target cells pulsed with each of candidate peptides were then established. The results demonstrated that the peptides are HLA-A24 or HLA-A2 restricted epitope peptides that can induce potent and specific immune responses against cells expressing WDRPUH on the surface. Further, it indicated that WDRPUH is strongly immunogenic and the epitopes thereof are effective targets for tumor immunotherapy.

[0011] Accordingly, it is an object of the present ~~invention~~disclosure to provide isolated peptides that bind to the HLA antigen, which peptides consist of WDRPUH (SEQ ID NO: 64) or a fragment of WDRPUH. Such peptides are expected to have CTL inducibility and can be used for inducing CTLs in ex vivo or for administration to a subject for inducing immune responses against cancers such as hepatocellular carcinoma. The peptides may be nonapeptides or decapeptides preferably consisting of the amino acid sequence selected from among SEQ ID NOs: 1, 2, 3, 4, 16, 17, 30, 31, 34, 36, 37, 40, 41, 45, 49, 55, 57 and 61, which show strong CTL inducibility.

[0011a] In one aspect, there is provided an isolated peptide of less than 15 amino acids, wherein the peptide is selected from the group consisting of:

(a) a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 1, 3, 4, 16, 17, 30, 31, 34, 36, 37, 40, 41, 45, 49, 55, 57 and 61; and

(b) a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:2, 1, 3, 4, 16, 17, 30, 31, 34, 36, 37, 40, 41, 45, 49, 55, 57 and 61 in which 1 or 2 amino acid(s) are substituted and/or added, and wherein the peptide has cytotoxic T lymphocyte (CTL) inducibility.

[0012] In addition, the present ~~invention~~disclosure contemplates modified peptides, having an amino acid sequence of SEQ ID NOs: 1, 2, 3, 4, 16, 17, 30, 31, 34, 36, 37, 40, 41, 45, 49, 55, 57 and 61 wherein one, two or more amino acids are substituted, inserted, deleted or added, so long as the modified peptides retain the original CTL inducibility.

[0013] It is a further object of the present ~~invention~~disclosure to provide isolated polynucleotides encoding any of the peptides of the present ~~invention~~disclosure. These polynucleotides can be used for inducing antigen-expressing cells (APCs) with CTL inducibility or for administration to a subject to induce immune responses against the present peptides and thus finally against cancers.

[0013a] In another aspect, there is provided an isolated APC that presents on its surface a complex of an HLA antigen and any one of the peptide(s) described herein. In another aspect, there is provided an isolated CTL that targets any one of the peptide(s) described herein.

[0014] When administered to a subject, the present peptides are presented on the surface of APCs and then induce CTLs targeting the respective peptides. Therefore, it is an object of the present ~~invention~~disclosure to provide agents containing any of the peptides or polynucleotides of the present ~~invention~~disclosure for inducing CTLs. These agents containing any of the peptides or polynucleotides of the present ~~invention~~disclosure can be used for the treatment and/or prophylaxis of cancers, such as hepatocellular carcinoma, and/or the prevention of postoperative recurrence thereof. Thus, it is yet another object of the present ~~invention~~disclosure to provide pharmaceutical agents for the treatment and/or prophylaxis of cancers, and/or prevention of postoperative recurrence thereof, which contains any of the peptides or polynucleotides of the present ~~invention~~disclosure. The present agents or pharmaceutical agents may also contain, as the active ingredient, APCs or exosomes which present any of the present peptides instead of or in addition to the present peptides or polynucleotides. In another aspect, there is provided a composition when used to induce a CTL *in vitro*, wherein the composition contains one or more peptide(s) described herein, or one or more polynucleotide(s) encoding any one of the peptide(s) described herein.

[0015] The peptides or polynucleotides of the present ~~invention~~disclosure have the ability to induce APCs which present, on its surface, a complex of an HLA antigen and the present peptide. For example, the induction can be achieved by contacting APCs derived from a subject with a peptide of the present ~~invention~~disclosure or introducing a polynucleotide encoding a peptide of the present ~~invention~~disclosure into APCs. Such APCs have high CTL inducibility against the target peptides and are useful for cancer immunotherapy.

In another aspect, there is provided an *in vitro* method for inducing an APC with CTL inducibility, wherein the method comprises a step selected from:

(a) contacting an APC with any one of the peptide(s) described herein; and

(b) introducing a polynucleotide encoding any one of the peptide(s) described herein into an APC.

Therefore, it is a further object of the present ~~invention~~disclosure to provide methods for inducing APCs with CTL inducibility and APCs obtained by the methods.

[0016] It is yet another object of the present ~~invention~~disclosure to provide methods for inducing CTLs, which methods contain the step of co-culturing CD8-positive cells with APCs or exosomes presenting a peptide of the present ~~invention~~disclosure on its surface or the step of introducing a polynucleotide into a T cell, which polynucleotide encodes a T cell receptor (TCR) subunit polypeptide binding to a peptide of the present ~~invention~~disclosure. The CTLs obtained by the methods are useful for treating and/or preventing cancers, such as hepatocellular carcinoma. In another aspect, there is provided an *in vitro* method for inducing a CTL, wherein the method comprises the step selected from:

(a) co-culturing CD8-positive T cells with APCs, which present on its surface a complex of an HLA antigen and any one of the peptide(s) described herein;

(b) co-culturing CD8-positive T cells with exosomes, which present on its surface a complex of an HLA antigen and any one of the peptide(s) described herein; and

(c) introducing a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide binding to any one of the peptide(s) described herein into a T cell.

Therefore, it is a further object of the present ~~invention~~disclosure to provide CTLs obtained by any of the present methods.

[0017] Another object of the present ~~invention~~disclosure to provide methods for inducing immune response against cancers, which methods contain the step of administering an agent containing any of the WDRPUH polypeptides, polynucleotides encoding WDRPUH polypeptides, exosomes or the APCs presenting WDRPUH polypeptides of the present ~~invention~~disclosure.

[0018] The present ~~invention~~disclosure find use for application to any diseases related to WDRPUH over-expression including, but are not limited to, cancer, particularly hepatocellular carcinoma. In another aspect, there is provided use of at least one component selected from the group consisting of (a) to (d) below:

(a) any one of the peptide(s) described herein;

(b) the polynucleotide encoding any one of the peptide(s) described herein;

(c) the APC described herein;

(d) the CTL described herein,

in the manufacture of a pharmaceutical composition for the treatment and/or prophylaxis of cancers expressing WDRPUH, and/or prevention of postoperative recurrence thereof.

[0019] It is to be understood that both the foregoing summary of the ~~invention~~disclosure and the

following detailed description are of exemplified embodiments, and not restrictive of the ~~invention~~disclosure or other alternate embodiments of the ~~invention~~disclosure.

Brief Description of Drawings

[0020] 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.

[fig.1]Figure 1 includes a series of photographs depicting the results of IFN-gamma ELISPOT assay on CTLs that were induced with peptides derived from WDRPUH. The CTLs in well numbers #3 and #6 stimulated with WDRPUH-A24-9-40 (SEQ ID NO: 1) (a), #8 with WDRPUH-A24-9-314 (SEQ ID NO: 2) (b), #2 and #6 with WDRPUH-A24-9-509 (SEQ ID NO: 3) (c), #1, #2 and #5 with WDRPUH-A24-9-339 (SEQ ID NO: 4) (d), #2, #3, #4, #6, #7 and #8 with WDRPUH-A24-10-409 (SEQ ID NO: 16) (e) and #5, #6 and #8 with WDRPUH-A24-10-40 (SEQ ID NO: 17) (f) showed potent IFN-gamma production as compared with control, respectively. The cells in the wells denoted with a rectangular box were expanded to establish CTL lines. In the figure, "+" indicates that the cells in the wells were pulsed with appropriate peptides, and "-" indicates that the cells had not been pulsed with any peptides.

[fig.2]Figure 2 includes a series of line graphs depicting IFN-gamma production of CTL lines stimulated with WDRPUH-A24-9-40 (SEQ ID NO: 1) (a), WDRPUH-A24-9-314 (SEQ ID NO: 2) (b), WDRPUH-A24-9-509 (SEQ ID NO: 3) (c), WDRPUH-A24-9-339 (SEQ ID NO: 4) (d), WDRPUH-A24-10-409 (SEQ ID NO: 16) (e) and WDRPUH-A24-10-40 (SEQ ID NO: 17) (f) with IFN-gamma ELISA assay. CTL lines established by stimulation with each of the peptides showed potent IFN-gamma production as compared with control. In the figure, "+" indicates that the cells in the wells were pulsed with appropriate peptides, and "-" indicates that the cells had not been pulsed with any peptides.

[fig.3]Figure 3 is composed of a line graph depicting specific CTL activity against target cells that exogenously express WDRPUH and HLA-A*2402. COS7 cells transfected with only HLA-A*2402 or with only the full length WDRPUH gene were prepared as control. The CTL line established with WDRPUH-A24-9-314 (SEQ ID NO: 2) showed specific CTL activity against COS7 cells transfected with both WDRPUH and HLA-A*2402 (black lozenge). In contrast, no significant specific CTL activity was detected against target cells expressing either HLA-A*2402 (triangle) or WDRPUH (circle).

[fig.4a-h]Figure 4a-h includes a series of photographs depicting the results of IFN-gamma ELISPOT assay on CTLs that were induced with peptides derived from

Claims

[Claim 1] An isolated peptide binding to an HLA antigen and having cytotoxic T lymphocyte (CTL) inducibility, wherein the peptide consists of the amino acid sequence of SEQ ID NO: 64 or is a fragment thereof.

[Claim 2] The isolated peptide of claim 1, wherein the HLA antigen is HLA-A24 or HLA-A2.

[Claim 3] The isolated peptide of claim 1 or 2, which is a nonapeptide or a decapeptide.

[Claim 4] The isolated peptide of any one of claims 1 to 3, which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 1, 3, 4, 16, 17, 30, 31, 34, 36, 37, 40, 41, 45, 49, 55, 57 and 61.

[Claim 5] The isolated peptide of claim 4, which consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 1, 3, 4, 16, 17, 30, 31, 34, 36, 37, 40, 41, 45, 49, 55, 57 and 61 wherein 1, 2, or several amino acid(s) are substituted, deleted, inserted and/or added.

[Claim 6] The peptide of claim 5, which consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, 16 and 17, wherein the peptide, when the HLA antigen is HLA-A24, has one or both of the following characteristics:
(a) the second amino acid from the N-terminus is selected from the group of phenylalanine, tyrosine, methionine and tryptophan; and
(b) the C-terminal amino acid is selected from the group of phenylalanine, leucine, isoleucine, tryptophan and, methionine.

[Claim 7] The peptide of claim 5, which consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 30, 31, 34, 36, 37, 40, 41, 45, 49, 55, 57 and 61, wherein the peptide, when the HLA antigen is HLA-A2, has one or both of the following characteristics:
(a) the second amino acid from the N-terminus is selected from the group of leucine and methionine; and
(b) the C-terminal amino acid is selected from the group of valine and leucine.

[Claim 8] An isolated polynucleotide encoding a peptide of any one of claims 1 to 7.

[Claim 9] An agent for inducing CTL, wherein the agent contains one or more peptide(s) of any one of claims 1 to 7, or one or more polynucleotide(s) of claim 8.

[Claim 10] A pharmaceutical agent for the treatment and/or prophylaxis of cancers,

and/or the prevention of postoperative recurrence thereof, wherein the agent comprises one or more peptide(s) of any one of claims 1 to 7, or one or more polynucleotide(s) of claim 8.

[Claim 11] The pharmaceutical agent of claim 10, formulated for the administration to a subject whose HLA antigen is HLA-A24 or HLA-A2.

[Claim 12] A method for inducing an antigen-presenting cell (APC) with CTL inducibility, wherein the method comprises a step selected from:
(a) contacting an APC with a peptide of any one of claims 1 to 7 in vitro, .ex vivo or in vivo; and
(b) introducing a polynucleotide encoding a peptide of any one of claims 1 to 7 into an APC.

[Claim 13] A method for inducing CTL, wherein the method comprises the step selected from:
(a) co-culturing CD8-positive T cells with APCs, which present on its surface a complex of an HLA antigen and a peptide of any one of claims 1 to 7;
(b) co-culturing CD8-positive T cells with exosomes, which present on its surface a complex of an HLA antigen and a peptide of any one of claims 1 to 7; and
(c) introducing a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide binding to a peptide of any one of claims 1 to 7 into a T cell.

[Claim 14] An isolated APC that presents on its surface a complex of an HLA antigen and a peptide of any one of claims 1 to 7.

[Claim 15] The APC of claim 14, which is induced by the method of claim 12.

[Claim 16] An isolated CTL that targets any peptide of any one of claims 1 to 7.

[Claim 17] The CTL of claims 16, which is induced by the method of claim 13.

[Claim 18] A method of inducing an immune response against a cancer in a subject, said method comprising the steps of administering to the subject an agent comprising a peptide of any one of claims 1 to 7, an immunologically active fragment thereof, or a polynucleotide encoding the peptide or the fragment.

CLAIMS:

1. An isolated peptide of less than 15 amino acids, wherein the peptide is selected from the group consisting of:

(a) a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 1, 3, 4, 16, 17, 30, 31, 34, 36, 37, 40, 41, 45, 49, 55, 57 and 61; and

(b) a peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:2, 1, 3, 4, 16, 17, 30, 31, 34, 36, 37, 40, 41, 45, 49, 55, 57 and 61 in which 1 or 2 amino acid(s) are substituted and/or added, and wherein the peptide has cytotoxic T lymphocyte (CTL) inducibility.

2. The peptide of Claim 1, wherein the peptide 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: 2, 1, 3, 4, 16 or 17 is substituted with an amino acid selected from the group consisting of phenylalanine, tyrosine, methionine and tryptophan; and

(b) the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 2, 1, 3, 4, 16 or 17 is substituted with an amino acid selected from the group consisting of phenylalanine, leucine, isoleucine, tryptophan and methionine.

3. The peptide of Claim 1, wherein the peptide 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:30, 31, 34, 36, 37, 40, 41, 45, 49, 55, 57 or 61 is substituted with leucine or methionine; and

(b) the C-terminal amino acid of the amino acid sequence of SEQ ID NO:30, 31, 34, 36, 37, 40, 41, 45, 49, 55, 57 or 61 is substituted with valine or leucine.

4. The peptide of Claim 1, wherein the peptide consists of the amino acid sequence selected from the group consisting of SEQ ID NOs:2, 1, 3, 4, 16, 17, 30, 31, 34, 36, 37, 40, 41, 45, 49, 55, 57 and 61.

5. A composition when used to induce a CTL *in vitro*, wherein the composition contains one or more peptide(s) of any one of Claims 1 to 4, or one or more polynucleotide(s) encoding the peptide of any one of Claims 1 to 4.

6. An *in vitro* method for inducing an APC with CTL inducibility, wherein the method comprises a step selected from:

(a) contacting an APC with the peptide of any one of Claims 1 to 4; and

(b) introducing a polynucleotide encoding the peptide of any one of Claims 1 to 4 into an APC.

7. An *in vitro* method for inducing a CTL, wherein the method comprises the step selected from:

(a) co-culturing CD8-positive T cells with APCs, which present on its surface a complex of an HLA antigen and the peptide of any one of Claims 1 to 4;

(b) co-culturing CD8-positive T cells with exosomes, which present on its surface a complex of an HLA antigen and the peptide of any one of Claims 1 to 4; and

(c) introducing a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide binding to the peptide of any one of Claims 1 to 4 into a T cell.

8. 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.

9. The APC of Claim 8, which is induced by the method of Claim 6.

10. An isolated CTL that targets the peptide of any one of Claims 1 to 4.

11. The CTL of Claim 10, which is induced by the method of Claim 7.

12. Use of at least one component selected from the group consisting of (a) to (d) below:

(a) the peptide of any one of Claims 1 to 4;

(b) the polynucleotide encoding the peptide of any one of Claims 1 to 4;

(c) the APC of Claim 8 or 9;

(d) the CTL of Claim 10 or 11,

in the manufacture of a pharmaceutical composition for the treatment and/or prophylaxis of cancers expressing WDRPUH, and/or prevention of postoperative recurrence thereof.