Summary

[0008] The present disclosure is based, at least in part, on the discovery of the 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. Recognizing that HJURP (SEQ ID NO: 50), typically encoded by the gene of GenBank Accession No. NM_018410 (SEQ ID NO: 49) has been identified as up-regulated in cancers, including, but not limited to, acute myeloid leukemia (AML), bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, chronic myeloid leukemia (CML), colorectal cancer, esophagus 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, the present disclosure focuses on HJURP as a candidate for the target of cancer/tumor immunotherapy, more particularly novel HJURP epitope peptides that may serve as suitable immunotherapeutic targets.

[0009] To that end, the present disclosure is directed, at least in part, to the identification of specific epitope peptides among the gene products of HJURP that possess the ability to induce CTLs specific to HJURP. 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 HJURP. CTL lines were then established with specific cytotoxicity against the HLA-A24- or HLA-A2-positive target cells pulsed with each of candidate peptides. The results herein demonstrate that these peptides are HLA-A24 or HLA-A2 restricted epitope peptides that may induce potent and specific immune responses against cells expressing HJURP. These results further demonstrate that HJURP is strongly immunogenic and that the epitopes thereof are effective targets for caner/tumor immunotherapy.

[0010] Accordingly, it is an object of the present disclosure to provide isolated peptides binding to HLA antigen, derived from HJURP (SEQ ID NO: 50) and the immunologically active fragments thereof. Such 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 examples of which include, but are not limited to, AML, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophagus 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 nonapeptides or decapeptides, and more preferably, a nonapeptide or decapeptide having an amino acid sequence selected from among SEQ ID

NOs: 2 to 24 and 26 to 48. Peptides having an amino acid sequence selected from among SEQ ID NOs: 3, 4, 7, 18, 23, 26, 27, 30, 31, 32, 35, 37, 38 and 43 showed strong CTL inducibility and thus are particularly preferred.

The present disclosure also contemplates modified peptides having an amino acid sequence selected from among SEQ ID NOs: 2 to 24 and 26 to 48, wherein one, two or more amino acids are substituted deleted or added, so long as the modified peptides retain the requisite original CTL inducibility.

The present disclosure further encompasses isolated polynucleotides encoding any peptides of the present disclosure. These polynucleotides can be used to induce or prepare APCs with CTL inducibility or, like the above-described peptides of the present disclosure, 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 agents, compositions or substances that include or incorporate any peptides or polynucleotides of the present disclosure for inducing CTLs. Such agents, compositions and/or substances can be used for the treatment and/or prophylaxis and/or postoperative recurrence of cancers, examples of which include, but are not limited to, AML, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophagus 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. Thus, it is yet another object of the present disclosure to provide pharmaceutical agents, compositions or substances for the treatment and/or prophylaxis and/or prevention of postoperative recurrence of cancer that include or incorporate any peptides or polynucleotides of the present disclosure. Instead of or in addition to the present peptides or polynucleotides, the pharmaceutical agents, compositions or substances of the present disclosure may include as active ingredients APCs or exosomes that present any of the present peptides.

[0012] The peptides or polynucleotides of the present disclosure may be used to induce APCs that present on the surface a complex of an HLA antigen and a 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 into APCs. Such APCs have high CTL inducibility against target peptides and find use in cancer immunotherapy. Accordingly, the present disclosure encompasses the methods for inducing APCs with CTL inducibility as well as APCs obtained by such methods.

[0013] It is a further object of the present disclosure to provide a method for inducing CTL, 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 of introducing a gene that includes a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide binding to the present peptide. CTLs obtained by such methods find use in the treatment and/or prevention of cancers, examples of which include, but are not limited to, AML, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophagus 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, it is yet another object of present disclosure to provide CTLs obtained by the present methods.

[0014] It is yet another object of the present disclosure to provide methods for inducing an immune response against cancer in a subject in need thereof, such methods including the step of administering compositions or substances including the HJURP polypeptides or immunologically active fragments thereof, polynucleotides encoding HJURP polypeptides, exosomes or the APCs presenting HJURP polypeptides.

[0015] The applicability of the present disclosure extends to any of a number of diseases relating to or arising from HJURP overexpression, such as cancer, examples of which include, but are not limited to, AML, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, esophagus 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.

[0016] More specifically, the present disclosure provides followings:

[1] An isolated peptide consisting of the amino acid sequence of SEQ ID NO: 50 or an immunologically active fragment thereof, wherein said peptide binds an HLA antigen and has cytotoxic T lymphocyte (CTL) inducibility,

[2] The isolated peptide of [1], wherein the HLA antigen is HLA-A24,

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

[4] The isolated peptide of [1] or [2], wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 to 24,

[5] The isolated peptide of [1] or [3] , wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 26 to 48,

[6] An isolated peptide selected from the group consisting of:

(a) an isolated peptide that binds to an HLA antigen, has cytotoxic T lymphocytes (CTL) inducibility, and consists of the amino acid sequence of SEQ ID NO: 50 or an immunologically active fragment thereof,

(b) the isolated peptide of (a), wherein the HLA antigen is HLA-A24,

(c) the isolated peptide of (a) or (b), which comprises an amino acid sequence

ID NOs: 3, 4, 7, 18 and 23,
[28] The isolated peptide of any one of [1], [3], [5], [7], [9], and [10], wherein the peptide consists of the amino acid sequence selected from the group consisting of SEQ ID NOs: 26, 27, 30, 31, 32, 35, 37, 38 and 43,
[29] An exosome that presents a complex comprising any of the peptides of [1] to [10] and an HLA antigen, and
[30] A host cell transformed or transfected with an expression vector according to [25].
[0016a] In one aspect, there is provided an isolated peptide of less than 15 amino acids selected from the group consisting of:
(a) an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38, 3, 4, 7, 18, 23, 26, 27, 30, 31, 32, 35, 37 and 43, and
(b) an isolated peptide comprising an amino acid sequence modified by substituting, deleting, inserting or adding 1 or 2 amino acid residues to an amino acid sequence selected from the group consisting of SEQ ID NOs: 38, 3, 4, 7, 18, 23, 26, 27, 30, 31, 32, 35, 37 and 43, provided said modified peptide has the CTL inducibility.
[0016b] In another aspect, there is provided an isolated polynucleotide encoding the peptide as described herein.
[0016c] In another aspect, there is provided a composition comprising one or more peptide(s) as described herein, or one or more polynucleotide(s) as described herein.
[0016d] In another aspect, there is provided use of a composition as described herein in the manufacture of a medicament for the treatment and/or prophylaxis of cancer overexpressing HJURP, and/or the prevention of a postoperative recurrence thereof.
[0016e] In another aspect, there is provided a method for inducing an antigen-presenting cell (APC) having CTL inducibility, the method comprising a step selected from the group consisting of: (a) contacting an APC with the peptide as described herein in vitro or ex vivo, and (b) introducing a polynucleotide encoding the peptide as described herein into an APC.
[0016f] In another aspect, there is provided an in vitro method for inducing a CTL, the method comprising a step selected from the group consisting of: (a) co-culturing CD8 positive T cells with APCs that present on the surface a complex of an HLA antigen and the peptide as described herein; (b) co-culturing CD8 positive T cells with exosomes that presents on the surface a complex of an HLA antigen and the peptide as described herein; and (c) introducing a gene that comprises a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide binding to the peptide as described herein into a T cell.
[0016g] In another aspect, there is provided an isolated APC that presents on its surface a complex of an HLA antigen and the peptide as described herein.
[0016h] In another aspect, there is provided an isolated CTL that targets the peptide as described herein.

[0016i] In another aspect, there is provided use of a composition as described herein in the manufacture of a medicament for inducing an immune response against cancer overexpressing HJURP in a subject in need thereof.
[0016j] In another aspect, there is provided an antibody or immunologically active fragment thereof against the peptide as described herein.
[0016k] In another aspect, there is provided a vector comprising a polynucleotide encoding the peptide as described herein.
[0016l] In another aspect, there is provided a diagnostic kit comprising the peptide as described herein, the polynucleotide as described herein or the antibody as described herein.
[0016m] In another aspect, there is provided an exosome that presents a complex comprising the peptide as described herein and an HLA antigen.
[0017] It is to be understood that both the foregoing summary of the present disclosure and the following detailed description are of exemplified embodiments, and not restrictive of the present disclosure or other alternate embodiments of the present disclosure.
In addition to the above, other objects and features of the disclosure will become more fully apparent when the following detailed description is read in conjunction with the accompanying figures and examples. However, 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. In particular, while the disclosure is described herein with reference to a number of specific embodiments, it will be appreciated that the description is illustrative of the disclosure and is not constructed as limiting of the disclosure. Various modifications and applications may occur to those who are skilled in the art, without departing from the spirit and the scope of the disclosure, as described by the appended claims. Likewise, other objects, features, benefits and advantages of the present disclosure will be apparent from this summary and certain embodiments described below, and will be readily apparent to those skilled in the art. Such objects, features, benefits and advantages will be apparent from the above in conjunction with the accompanying examples, data, figures and all reasonable inferences to be drawn therefrom, alone or with consideration of the references incorporated herein.

Brief Description of Drawings

[0018] 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.
[0019] [Fig. 1]
Figure 1 is composed of a series of photographs, (a) - (f), depicting the results of IFN-gamma ELISPOT assays on CTLs that were induced with peptides derived from HJURP. The CTLs in well number #4 stimulated with HJURP-A24-9-28 (SEQ ID NO: 3) (a), in #4 with HJURP-A24-9-263 (SEQ ID NO: 4) (b), in #4 with HJURP-A24-9-408 (SEQ ID NO: 7) (c), in #6 with HJURP-A24-10-383 (SEQ ID NO: 18) (d) and in #4 with HJURP-A24-10-162 (SEQ ID NO: 23) (e), showed potent IFN-gamma

groups or characteristics in common: an aliphatic side-chain (G, A, V, L, I, P); a hydroxyl group containing side-chain (S, T, Y); a sulfur atom containing side-chain (C, M); a carboxylic acid and amide containing side-chain (D, N, E, Q); a base containing side-chain (R, K, H); and an aromatic group containing side-chain (H, F, Y, W). In addition, the following eight groups each contain amino acids that are conservative substitutions for one another:
1) Alanine (A), Glycine (G);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V);
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W);
7) Serine (S), Threonine (T); and
8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins 1984).

[0051]

Such conservatively modified peptides are also considered to be peptides of the present invention. However, the peptide of the present invention is not restricted thereto and may include non-conservative modifications, so long as the resulting modified peptide retains the CTL inducibility of the original peptide. Furthermore, the modified peptides should not exclude CTL inducible peptides of polymorphic variants, interspecies homologues, and alleles of HJURP.

[0052]

Amino acid residues may be inserted, substituted or added to the peptides of the present invention or, alternatively, amino acid residues may be deleted there from to achieve a higher binding affinity. To retain the requisite CTL inducibility, one preferably modifies (inserts, deletes, adds and/or substitutes) only a small number (for example, 1, 2 or several) or a small percentage of amino acids. Herein, the term "several" means 5 or fewer amino acids, for example, 4 or 3 or fewer. The percentage of amino acids to be modified may be 20% or less, for example, 15% or less, even more preferably 10% or less, for example 1 to 5%.

[0053]

When used in cancer immunotherapy, the present peptides are presented on the surface of a cell or exosome as a complex with an HLA antigen. Therefore, it is preferable to select peptides that not only induce CTLs but also possess high binding affinity to the HLA antigen. To that end, the peptides can be modified by substitution, insertion, and/or addition of the amino acid residues to yield a modified peptide having improved binding affinity. In addition to peptides that are naturally displayed, since the regularity of the sequences of peptides displayed by binding to HLA antigens has already been known (J Immunol 1994, 152: 3913; Immunogenetics 1995, 41: 178; J Immunol 1994, 155: 4307), modifications based on such regularity may be introduced into the immunogenic peptides of the present invention.

Claims

1. An isolated peptide of less than 15 amino acids selected from the group consisting of:
(a) an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 38, 3, 4, 7, 18, 23, 26, 27, 30, 31, 32, 35, 37 and 43, and
(b) an isolated peptide comprising an amino acid sequence modified by substituting, deleting, inserting or adding 1 or 2 amino acid residues to an amino acid sequence selected from the group consisting of SEQ ID NOs: 38, 3, 4, 7, 18, 23, 26, 27, 30, 31, 32, 35, 37 and 43, provided said modified peptide has the CTL inducibility.

2. The isolated 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: 38, 26, 27, 30, 31, 32, 35, 37 or 43 is substituted with an amino acid selected from the group consisting of leucine and methionine; and
(b) the C-terminal amino acid of the amino acid sequence of SEQ ID NO: 38, 26, 27, 30, 31, 32, 35, 37 or 43 is substituted with an amino acid selected from the group consisting of valine and leucine.

3. The isolated peptide of claim 1, wherein the peptide has one or both of the following characteristics:
(a) the second amino acid from N-terminus of the amino acid sequence of SEQ ID NO: 3, 4, 7, 18 or 23 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: 3, 4, 7, 18 or 23 is substituted with an amino acid selected from the group consisting of phenylalanine, leucine, isoleucine, tryptophan and methionine.

4. The isolated peptide of any one of claims 1 to 3, wherein said peptide is a nonapeptide or decapeptide.

5. The isolated peptide of claim 4, wherein said peptide consists of the amino acid sequences selected from the group consisting of SEQ ID NOs: 38, 26, 27, 30, 31, 32, 35, 37, 43, 3, 4, 7, 18 and 23.

6. An isolated polynucleotide encoding the peptide of any one of claims 1 to 5.

7. A composition comprising one or more peptide(s) of any one of claims 1 to 5, or one or more

polynucleotide(s) of claim 6.

8. Use of a composition of claim 7 in the manufacture of a medicament for the treatment and/or prophylaxis of cancer overexpressing HJURP, and/or the prevention of a postoperative recurrence thereof.

9. The use according to claim 8, wherein said pharmaceutical composition is formulated for the administration to a subject whose HLA antigen is HLA-A2 or HLA-A24.

10. The use according to claim 8 or 9, wherein said pharmaceutical composition is formulated for the treatment of cancer.

11. A method for inducing an antigen-presenting cell (APC) having CTL inducibility, the method comprising a step selected from the group consisting of:
(a) contacting an APC with the peptide of any one of claims 1 to 5 in vitro or ex vivo, and
(b) introducing a polynucleotide encoding the peptide of any one of claims 1 to 5 into an APC.

12. An in vitro method for inducing a CTL, the method comprising a step selected from the group consisting of:
(a) co-culturing CD8 positive T cells with APCs that present on the surface a complex of an HLA antigen and the peptide of any one of claims 1 to 5;
(b) co-culturing CD8 positive T cells with exosomes that presents on the surface a complex of an HLA antigen and the peptide of any one of claims 1 to 5; and
(c) introducing a gene that comprises a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide binding to the peptide of any one of claims 1 to 5 into a T cell.

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 5.

14. The APC of claim 13, which is induced by the method of claim 11.

15. An isolated CTL that targets the peptide of any one of claims 1 to 5.

16. The CTL of claim 15, which is induced by the method of claim 12.

17. Use of a composition of claim 7 in the manufacture of a medicament for inducing an immune

response against cancer overexpressing HJURP in a subject in need thereof.

18. An antibody or immunologically active fragment thereof against the peptide of any one of claims 1 to 5.

19. A vector comprising a polynucleotide encoding the peptide of any one of claims 1 to 5.

20. A diagnostic kit comprising the peptide of any one of claims 1 to 5, the polynucleotide of claim 6 or the antibody of claim 18.

21. An exosome that presents a complex comprising the peptide of any one of claims 1 to 5 and an HLA antigen.