described risk of immune escape of cancer cells attributable to deletion, mutation, or down-regulation of TAAs as a consequence of therapeutically driven immune selection.
On the other hand, Nei endonuclease VIII-like 3 (NEIL3) has been isolated as a member belonging to a class of DNA glycosylases homologous to the bacterial Fpg/Nei family (NPL 14/Bandaru et al., DNA Repair (Amst). 2002 Jul 17;1(7):517-29). These glycosylases initiate the first step in base excision repair by cleaving bases damaged by reactive oxygen species and introducing a DNA strand break via the associated lyase reaction. NEIL3 is likely to play a role in DNA repair mechanism, however, its relationship with carcinogenesis has not been elucidated.

Citation List

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[0004]

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Summary ~~of Invention~~

[0005]

The present ~~invention~~disclosure is based, at least in part, on the discovery of the applicable 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, NEIL3 (SEQ ID NO: 45 encoded by the gene of GenBank Accession No. NM_018248 (for example, SEQ ID NO: 44)) has been identified as up-regulated in cancers, such as bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, colorectal cancer, endometriosis, esophagus cancer, liver cancer, non-small cell lung cancer (NSCLC), osteosarcoma, pancreatic cancer, prostate cancer, renal carcinoma, small cell lung cancer (SCLC), soft tissue tumor, acute myeloid leukemia (AML) and chronic myeloid leukemia

(CML). Thus, NEIL3 is a candidate for the target of cancer/tumor immunotherapy.

[0006]

The present ~~invention~~disclosure is based, at least in part, on the identification of specific epitope peptides of the gene products of NEIL3 which possess the ability to induce CTLs specific to NEIL3. 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 NEIL3. CTL lines were then established with specific cytotoxicity against the HLA-A24 or HLA-A2 positive target cells pulsed with each of candidate peptides. These results demonstrate that these peptides are HLA-A24 or HLA-A2 restricted epitope peptides that may induce potent and specific immune responses against cells expressing NEIL3. Further, it indicated that NEIL3 is strongly immunogenic and the epitopes thereof are effective targets for caner/tumor immunotherapy.

[0007]

Accordingly, the present ~~invention~~disclosure provides isolated peptides binding to HLA antigen which consists of NEIL3 (SEQ ID NO: 45) or the immunologically active fragments thereof. These peptides are expected to have CTL inducibility and can be used to induce CTL ex vivo or to be administered to a subject for inducing immune responses against cancers such as bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, endometriosis, liver cancer, NSCLC, osteosarcoma, pancreatic cancer, SCLC and AML. Preferably, those peptides are nonapeptide or decapeptide, and more preferably, consisting of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 43. In particular, the peptides consisting of the amino sequence selected from the group consisting of SEQ ID NOs: 3, 4, 5, 6, 11, 15, 17, 21, 22, 24, 33, 35, 41 and 43 showed strong CTL inducibility.

[0008]

The peptides of the present ~~invention~~disclosure encompass those wherein one, two or more amino acids are substituted deleted or added, so long as the modified peptides retain the original CTL inducibility.

In one aspect, there is provided an isolated peptide of less than 15 amino acids selected from the group consisting of (i) and (ii) below:

(i) an isolated peptide of (a) or (b) below:

(a) an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 33, 35, 41 and 43;

(b) an isolated peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 33, 35, 41 and 43 in which 1 or 2 amino acid(s) are substituted, deleted, or added, wherein the peptide has CTL inducibility; and

(ii) an isolated peptide of (c) or (d) below:

(c) an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 3, 4, 6, 11, 15, 17, 21 and 22;

(d) an isolated peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 3, 4, 6, 11, 15, 17, 21 and 22 in which 1 or 2 amino acid(s) are substituted, deleted, or added, wherein the peptide has CTL inducibility.

Further, in another aspect, there is provided~~the present invention provides~~ isolated polynucleotides encoding any one of the peptides of the present ~~invention~~disclosure. These polynucleotides can be used for inducing or preparing APCs with CTL inducibility or to be administered to a subject for inducing immune responses against cancers as well as the present peptides.

[0009]

When administered to a subject, the present peptides are presented on the surface of APCs and then induce CTLs targeting the respective peptides. Therefore, according to an ~~aspect~~embodiment of the present ~~invention~~disclosure, compositions or substances including any peptides or polynucleotides of the present ~~invention~~disclosure for inducing CTLs are also provided. In one aspect, there is provided a composition comprising one or more peptide(s) as defined herein, or one or more polynucleotide(s) as defined herein. Furthermore, compositions or substances including any peptides or polynucleotides can be used to treating and/or prophylaxis of cancers, such as bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, colorectal cancer, endometriosis, esophagus cancer, liver cancer, NSCLC, osteosarcoma, pancreatic cancer, prostate

cancer, renal carcinoma, SCLC, soft tissue tumor, AML and CML, and/or preventing postoperative recurrence thereof. Thus, the present ~~invention~~disclosure also provides pharmaceutical compositions or substances for treating and/or prophylaxis of cancers, and/or preventing postoperative recurrence thereof, which includes any peptides or polynucleotides of the present ~~invention~~disclosure. In one aspect, there is provided a pharmaceutical composition comprising one or more peptide(s) as defined herein, or one or more polynucleotides as defined herein. The present pharmaceutical compositions or substances may include APCs or exosomes which present any of the present peptides instead of/in addition to the present peptides or polynucleotides as active ingredients.

In one aspect, there is provided the peptide as defined herein, polynucleotide as defined herein, or the composition as defined herein for use as a medicament.

In one aspect, there is provided the peptide as defined herein, polynucleotide as defined herein, or the composition as defined herein for use in the treatment or prevention of cancer and/or preventing postoperative recurrence thereof.

[0010]

The peptides or polynucleotides of the present ~~invention~~disclosure can 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 the present ~~invention~~disclosure into APCs. Such APCs have high CTL inducibility against target peptides and find use in cancer immunotherapy. Therefore, the present ~~invention~~disclosure encompasses the methods for inducing APCs with CTL inducibility and the APCs obtained by the methods.

In one aspect, there is provided an in vitro 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 as defined herein in vitro, and

(b) introducing a polynucleotide encoding the peptide as defined herein into an APC in vitro.

In one aspect, there is provided an isolated APC that presents on its surface a complex of an HLA antigen and the peptide as defined herein.

[0011]

The present ~~invention~~disclosure also provides a method for inducing CTL, which includes the step of co-culturing CD8 positive cells with APCs or exosomes presenting the peptide of the present ~~invention~~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. In one aspect, there is provided in vitro method for inducing CTL by method comprising a step selected from the group consisting of:

(a) co-culturing CD8 positive T cells with APCs, which presents on its surface a complex of an HLA antigen and the peptide as defined herein;

(b) co-culturing CD8 positive T cells with exosomes, which presents on its surface a complex of an HLA antigen and a peptide as defined herein; and

(c) introducing a gene that comprises a polynucleotide encoding a T cell receptor (TCR) subunit polypeptide binding to the peptide as defined herein into a T cell.

The CTLs obtained by the methods can find use for treating and/or preventing cancers, such as bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, colorectal cancer, endometriosis, esophagus cancer, liver cancer, NSCLC, osteosarcoma, pancreatic cancer, prostate cancer, renal carcinoma, SCLC, soft tissue tumor, AML and CML. Therefore, the present ~~invention~~disclosure encompasses the CTLs obtained by the present methods. In one aspect, there is provided an isolated CTL that targets the peptide as defined herein. In one aspect, there is provided an exosome that presents a complex comprising the peptide as defined herein and an HLA antigen. In one aspect, there is provided an antibody or fragment thereof against the peptide as defined herein. In one aspect, there is provided a vector comprising a nucleotide sequence encoding the peptides as defined herein. In one aspect, there is provided a host cell transformed or transfected with an expression vector as defined herein. In one aspect, there is provided a diagnostic kit comprising the peptide as defined herein, the polynucleotide as defined herein or the antibody as defined herein.

[0012]

Moreover, the present ~~invention~~disclosure provides methods for inducing immune response against cancers, which methods include the step of administering compositions or substances including the NEIL3 polypeptides or immunologically active fragments thereof, polynucleotides encoding NEIL3 polypeptides, exosomes or the APCs presenting NEIL3 polypeptides.

The present ~~invention~~disclosure also provides a method of diagnosing cancer, including, but not limited to, bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, colorectal cancer, endometriosis, esophagus cancer, liver cancer, NSCLC, osteosarcoma, pancreatic cancer, prostate cancer, renal carcinoma, SCLC, soft tissue tumor, AML and CML. The present ~~invention~~disclosure may be applied to any diseases relating to NEIL3 overexpression, such as cancer, exemplary cancers include bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, colorectal cancer, endometriosis, esophagus cancer, liver cancer, NSCLC, osteosarcoma, pancreatic cancer, prostate

Claims

[Claim 1] An isolated peptide binding to an HLA antigen and having cytotoxic T lymphocytes (CTL) inducibility, wherein the peptide consists of the amino acid sequence of SEQ ID NO: 45 or an immunologically active fragment thereof.

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

[Claim 3] The isolated peptide of claim 1 or 2, which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 4, 5, 6, 11, 15, 17, 21 and 22.

[Claim 4] The isolated peptide of any one of claims 1, 2, and 3, which consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 4, 5, 6, 11, 15, 17, 21 and 22, wherein 1, 2, or several amino acid(s) are substituted, deleted, or added.

[Claim 5] The isolated peptide of claim 4 which consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 4, 5, 6, 11, 15, 17, 21 and 22, wherein the peptide has one or both of the following characteristics:
(a) second amino acid from N-terminus is selected from the group consisting of leucine and methionine; and
(b) C-terminal amino acid is selected from the group consisting of valine and leucine.

[Claim 6] The isolated peptide of claim 1, wherein the HLA antigen is HLA-A24.

[Claim 7] The isolated peptide of claim 1 or 6, which comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 33, 35, 41 and 43.

[Claim 8] The isolated peptide of any one of claims 1, 6 and 7, which consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 33, 35, 41 and 43, wherein 1, 2, or several amino acid(s) are substituted, deleted, or added.

[Claim 9] The isolated peptide of claim 8, which consists of an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 33, 35, 41 and 43, wherein the peptide has one or both of the following characteristics:
(a) second amino acid from the N-terminus is selected from the group consisting of phenylalanine, tyrosine, methionine and tryptophan; and
(b) C-terminal amino acid is selected from the group consisting of phenylalanine, leucine, isoleucine, tryptophan and methionine.

[Claim 10] The isolated peptide of any one of claims 1 to 9, which is nonapeptide or decapeptide.

[Claim 11] An isolated polynucleotide encoding the peptide of any one of claims 1 to 10.

[Claim 12] A composition for inducing CTL, wherein the composition comprises one or more peptide(s) of any one of claims 1 to 10, or one or more polynucleotide(s) of claim 11.

[Claim 13] A pharmaceutical composition for treating and/or prophylaxis of cancers, and/or preventing postoperative recurrence thereof, wherein the composition comprises one or more peptide(s) of any one of claims 1 to 10, or one or more polynucleotides of claim 11.

[Claim 14] The pharmaceutical composition of claim 13, which is intended for the administration to a subject whose HLA antigen is HLA-A24 or HLA-A2.

[Claim 15] The pharmaceutical composition of claim 13 or 14, which is intended for treating cancer.

[Claim 16] 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 10 in vitro, ex vivo or in vivo, and
(b) introducing a polynucleotide encoding the peptide of any one of claims 1 to 10 into an APC.

[Claim 17] A method for inducing CTL by method comprising a step selected from the group consisting of:
(a) co-culturing CD8 positive T cells with APCs, which presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 10;
(b) co-culturing CD8 positive T cells with exosomes, which presents on its surface a complex of an HLA antigen and a peptide of any one of claims 1 to 10; 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 10 into a T cell.

[Claim 18] An isolated APC that presents on its surface a complex of an HLA antigen and the peptide of any one of claims 1 to 10.

[Claim 19] The APC of claim 18, which is induced by the method of claim 16.

[Claim 20] An isolated CTL that targets any of the peptides of claims 1 to 10.

[Claim 21] The CTL of claims 20, which is induced by the method of claim 17.

[Claim 22] A method of inducing an immune response against cancer in a subject comprising administering to the subject a composition comprising a peptide of claims 1 to 10, an immunologically active fragment thereof, or a polynucleotide encoding the peptide or the fragment.

[Claim 23] An exosome that presents a complex comprising any of the peptides of claims 1 to 10 and an HLA antigen.

[Claim 24] An antibody or fragment thereof against any of the peptides of claims 1 to 10.

[Claim 25] A vector comprising a nucleotide sequence encoding any of the peptides of claims 1 to 10.

[Claim 26] A host cell transformed or transfected with an expression vector according to claim 25.

[Claim 27] A diagnostic kit comprising any of the peptides of claims 1 to 10, the nucleotide of claim 11 or the antibody of claim 24.

[Claim 28] A method for diagnosing cancer, said method comprising the steps of:
(a) determining the expression level of a NEIL3 gene in a subject-derived biological sample by any one of the method select from the group consisting of:
(i) detecting the mRNA including the sequence of NEIL3,
(ii) detecting the protein including the amino acid sequence of NEIL3, and
(iii) detecting the biological activity of the protein including the amino acid sequence of NEIL3; and
(b) correlating an increase in the expression level determined in step (a) as compared to a normal control level of the gene to the presence of cancer.

[Claim 29] The method of claim 28, wherein the expression level determined in step (a) is at least 10% greater than the normal control level.

[Claim 30] The method of claim 28, wherein the cancer is selected from group of bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, colorectal cancer, endometriosis, esophagus cancer, liver cancer, NSCLC, osteosarcoma, pancreatic cancer, prostate cancer, renal carcinoma, SCLC, soft tissue tumor, AML and CML.

[Claim 31] The method of claim 28, wherein the expression level determined in step (a) is determined by detecting hybridization of a probe to a gene transcript of the gene.

[Claim 32] The method of claim 28, wherein the expression level determined in step (a) is determined by detecting the binding of an antibody against

the protein of NEIL3.

[Claim 33] The method of claim 28, wherein the subject-derived biological sample comprises biopsy, sputum, blood, pleural effusion or urine.

[Claim 34] The method of claim 28, wherein the NEIL3 gene encodes a polypeptide having the amino acid sequence shown in SEQ ID NO: 45.

[Claim 35] The method of claim 28, wherein the amino acid of NEIL3 is SEQ ID NO: 45.

[Claim 36] A kit comprising a detection reagent which binds the transcription or translation product of the NEIL3.

CLAIMS

1. An isolated peptide of less than 15 amino acids selected from the group consisting of (i) and (ii) below:

   (i) an isolated peptide of (a) or (b) below:
   (a) an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 33, 35, 41 and 43;
   (b) an isolated peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 33, 35, 41 and 43 in which 1 or 2 amino acid(s) are substituted, deleted, or added, wherein the peptide has CTL inducibility; and

   (ii) an isolated peptide of (c) or (d) below:
   (c) an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 3, 4, 6, 11, 15, 17, 21 and 22;
   (d) an isolated peptide consisting of an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 3, 4, 6, 11, 15, 17, 21 and 22 in which 1 or 2 amino acid(s) are substituted, deleted, or added, wherein the peptide has 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: 24, 33, 35, 41 or 43 is substituted with the 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: 24, 33, 35, 41 or 43 is substituted with the amino acid selected from the group consisting of phenylalanine, leucine, isoleucine, tryptophan and methionine.

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: 5, 3, 4, 6, 11, 15, 17, 21 or 22 is substituted with the 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: 5, 3, 4, 6, 11, 15, 17, 21 or 22 is substituted with the amino acid selected from the group consisting of valine and leucine.

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

5. The isolated peptide of claim 1 or 4, wherein the peptide consists of the amino acid sequence selected from the group consisting of SEQ ID NO: 5, 3, 4, 6, 11, 15, 17, 21, 22, 24, 33, 35, 41 and 43.

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. A pharmaceutical composition comprising one or more peptide(s) of any one of claims 1 to 5, or one or more polynucleotides of claim 6.

9. The peptide according to any one of claims 1 to 5, polynucleotide according to claim 6, or the composition according to claim 7 or 8 for use as a medicament.

10. The peptide according to any one of claims 1 to 5, polynucleotide according to claim 6, or the composition according to claim 7 or 8 for use in the treatment or prevention of cancer and/or preventing postoperative recurrence thereof.

11. The peptide, polynucleotide, or composition for use in the treatment or prevention of cancer and/or preventing postoperative recurrence thereof according to claim 10 for the administration to a subject whose HLA antigen is HLA-A24 or HLA-A2.

12. An in vitro 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 5 in vitro, and

(b) introducing a polynucleotide encoding the peptide of any one of claims 1 to 5 into an APC in vitro.

13. An in vitro method for inducing CTL by method comprising a step selected from the group consisting of:

(a) co-culturing CD8 positive T cells with APCs, which presents on its 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, which presents on its surface a complex of an HLA antigen and a 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.

14. 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.

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

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

17. The CTL of claim 16, which is induced by the method of claim 13.

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

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

20. A vector comprising a nucleotide sequence encoding the peptides of any one of claims 1 to 5.

21. A host cell transformed or transfected with an expression vector according to claim 20.

22. A diagnostic kit comprising the peptide of any one of claims 1 to 5, the polynucleotide of claim 6 or the antibody of claim 19.