expressed in cancer (Lin YM, et al., Oncogene. 2002 Jun 13;21:4120-8.; Kitahara O, et al., Cancer Res. 2001 May 1;61:3544-9.; Suzuki C, et al., Cancer Res. 2003 Nov 1;63:7038-41.; Ashida S, Cancer Res. 2004 Sep 1;64:5963-72.; Ochi K, et al., Int J Oncol. 2004 Mar;24(3):647-55.; Kaneta Y, et al., Int J Oncol. 2003 Sep;23:681-91.; Obama K, Hepatology. 2005 Jun;41:1339-48.; Kato T, et al., Cancer Res. 2005 Jul 1;65:5638-46.; Kitahara O, et al., Neoplasia. 2002 Jul-Aug;4:295-303.; Saito-Hisaminato A et al., DNA Res 2002, 9: 35-45.). Of these, MPHOSPH1 (in house No. C2093) and DEPDC1 (in house No. B5860N) were identified genes over-expressed in various cancers. In particular, MPHOSPH1 was identified as over-expressed in bladder cancer, breast cancer, cervical cancer, cholangincellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, soft tissue tumor. Similarly, DEPDC1 was identified as over-expressed in bladder cancer, breast cancer, cervical cancer, cholangincellular carcinoma, CML, NSCLC, lymphoma, osteosarcoma, prostate cancer, SCLC, soft tissue tumor

[0008] MPHOSPH1 was previously identified as one of the proteins specifically phosphorylated at the G2/M transition and characterized as a plus-end-directed kinesin related protein (Abaza A et al., J Biol Chem 2003, 278: 27844-52.). More particularly, MPHOSPH1 has been previously documented to be a plus-end-directed molecular motor that plays a crucial role in cytokinesis, and accumulates in the midzone of the spindle during anaphase to telophase in HeLa cells (Abaza A et al., J Biol Chem 2003, 278: 27844-52; Kamimoto T et al., J Biol Chem 2001, 276: 37520-8). The MPHOSPH1 cDNA encodes a 1780-amino acid protein that is composed of three domains: an NH2-kinasin motor domain, a central coiled coil-stalk domain, and a C-globular tail domain. Together, this data suggests that MPHOSPH1 is an NH2-type kinesin-related protein.

[0009] As for DEPDC1, its function remains unclear. The DEP domain contained in this protein is also found in Dishevelled, Egl-10, and Pleckstrin. The DEP domain in Drosophila dishevelled plays an essential role in rescue planar polarity defects and induces JNK signaling; nevertheless, its function in Humans has not yet been clarified. However, as disclosed in PCT/JP2006/302684, DEPDC1 siRNAs can suppress the growth of cancer cells. These results demonstrate that DEPDC1 plays an important role in growth of most cancer cells.

[0010] Summary ~~of the Invention~~

As noted above, MPHOSPH1 (M-phase phosphoprotein 1), and DEPDC1 (DEP domain containing 1) have been identified as up-regulated in various cancers. More particularly, the genes were identified using gene expression profiling with a genome-wide cDNA microarray. As discussed above, expression of MPHOSPH1 and DEPDC1 has been shown to be specifically up-regulated in various tumor cells, including lung

cancer and bladder cancer. As described in Table 1, MPHOSPH1 expression was shown to be validly elevated in 30 out of 31 bladder cancers, 8 out of 36 breast cancers, 18 out of 18 cervical cancers, 5 out of 17 cholangincellular carcinomas, 25 out of 31 CMLs, 6 out of 11 colorectal cancers, 6 out of 14 gastric cancers, 5 out of 5 NSCLCs, 7 out of 7 lymphomas, 6 out of 10 osteosarcomas, 7 out of 22 prostate cancers, 10 out of 18 renal carcinomas and 15 out of 21soft tissue tumors. At the same time, DEPDC1 expression was shown to be validly elevated in 23 out of 25 bladder cancers, 6 out of 13 breast cancers, 12 out of 12 cervical cancers, 6 out of 6 cholangin-cellular carcinomas, 3 out of 4 CMLs 2 out of 4 colorectal cancers, 6 out of 6 NSCLCs, 7 out of 7 lymphomas, 10 out of 14 osteosarcomas, 11 out of 24 prostate cancers, 14 out of 14 SCLCs and 22 out of 31 soft tissue tumors as described in Table 1.

In one aspect, the present invention refers to an isolated peptide having cytotoxic T cell inducibility, wherein said peptide is derived from the amino acid sequence of SEQ ID NO:4 or 6, wherein the peptide is less than 15 amino acids, wherein the peptide comprises the amino acid sequence selected from the group consisting of SEQ ID NOs:12, 192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 249, 253, 254 and 255. In another aspect, the present invention refers to an isolated peptide of less than 15 amino acids, wherein the peptide comprises the amino acid sequence selected from the group consisting of (a) the amino acid sequence of SEQ ID NO:12; and (b) the amino acid sequence of SEQ ID NO:12, in which 1, 2, or several amino acids are substituted, deleted, or added, wherein the peptide has cytotoxic T cell inducibility. In yet another aspect, the present invention refers to an isolated peptide of less than 15 amino acids, wherein the peptide comprises the amino acid sequence selected from the group consisting of (a) the amino acid sequence selected from the group consisting of: SEQ ID NOs:192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 249, 253, 254 and 255; and (b) the amino acid sequence selected from the group consisting of SEQ ID NOs:192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 249, 244, 253, 254 and 255, in which 1, 2, or several amino acids are substituted, deleted, or added, wherein the peptide has cytotoxic T cell inducibility. In a further aspect, the present invention refers to an isolated peptide consisting of the amino acid sequence selected from the group consisting of SEQ ID NOs:12, 192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 249, 253, 254 and 255. In yet another aspect, the present invention refers to a vector comprising DNA which encodes the peptide as described herein.

In another aspect, the present invention refers to a pharmaceutical composition for treating or preventing a disease associated with over-expression of the genes of SEQ ID NO:3 and/or 5, said composition comprising one or more peptides as described herein. In one aspect, the present invention refers to an exosome that presents on its surface a complex comprising a peptide as described herein and an HLA antigen. In another aspect, the present invention refers to an in vitro method of inducing antigen-presenting cells having cytotoxic T cell inducibility, said method comprising the step of (a) contacting an antigen-presenting cell with a peptide as described herein or (b) transferring a gene comprising a polynucleotide encoding a peptide as described herein to an antigen-presenting cell. In a further aspect, the present invention refers to an in vitro method of inducing cytotoxic T cells, said method comprising the step of contacting $CD8^+$ T cells with antigen-presenting cells induced by the method as described herein. In yet another aspect, the present invention refers to an isolated cytotoxic T cell, which is induced by the method as described herein or which is transduced with the nucleic acids encoding the TCR subunits polypeptides binding with a peptide as described herein in the context of HLA-A24 or HLA-A2. In another aspect, the present invention refers to an isolated antigen-presenting cell, which comprises a complex formed between an HLA antigen and a peptide as described herein. In one aspect, the present invention refers a vaccine for inhibiting proliferation of cells expressing either or both of the genes of SEQ ID NO:3 and 5, wherein the vaccine comprises a peptide as described herein as an active ingredient.

[0011] The present ~~invention~~disclosure is based, at least in part, on the identification of specific epitope peptides of the gene products of these genes (MPHOSPH1 and DEPDC1) which possess the ability to induce cytotoxic T lymphocytes (CTLs) specific to the corresponding molecules. As discussed in detail below, Peripheral Blood Mononuclear Cells (PBMC) of healthy donor were stimulated using HLA-A*2402 and HLA-A*0201 binding candidate peptides derived from MPHOSPH1 or DEPDC1. CTL clones and/or lines were then established with specific cytotoxicity against the HLA-A24 or HLA-A2 positive target cells pulsed with each of the candidate peptides. These results demonstrate that these peptides are HLA-A24 or HLA-A2 restricted epitope peptides that can induce potent and specific immune responses against cells expressing MPHOSPH1 or DEPDC1.

[0012] Accordingly, the present ~~invention~~disclosure provides methods for treating or preventing a disease associated with the over-expression of MPHOSPH1 and/or DEPDC1, e.g. cancer. Such methods involves the step of administering to a subject in need thereof a MPHOSPH1 and/or DEPDC1 polypeptide of the invention. Administration of such peptide(s) results in the induction of anti-tumor immunity. Thus, the present ~~invention~~disclosure

provides methods for inducing anti-tumor immunity in a subject, such methods involving the step of administering to the subject a MPHOSPH1 and/or DEPDC1 polypeptide, as well as pharmaceutical compositions for treating or preventing a disease associated with the over-

expression of MPHOSPH1 and/or DEPDC1, e.g cancer, that include the MPHOSPH1 and/or DEPDC1 polypeptides. Exemplary cancers include, but are not limited to, bladder cancer, breast cancer, cervical cancer, cholangincellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC, and soft tissue tumor.

[0013] That is, the present ~~application~~ disclosure includes the following ~~embodiments~~examples, and any combinations thereof.

ducibility comprising the step of contacting an antigen-presenting cell with a peptide of any one of [1] to [7].

[18] A method of inducing cytotoxic T cells by contacting a T cell with a peptide of any one of [1] to [7].

[19] A method of inducing antigen-presenting cells having high cytotoxic T cell inducibility, said method comprising the step of transferring a gene comprising a polynucleotide encoding a peptide of any one of [1] to [7] to an antigen-presenting cell.

[20] An isolated cytotoxic T cell, which is induced by contacting a T cell with a peptide of any one of claims 1 to 7 or which is transduced with the nucleic acids encoding the TCR subunits polypeptides binding with a peptide of any one claims 1 to 7 in the context of HLA-A24 or HLA-A2.

[21] An antigen-presenting cell, which comprises a complex formed between an HLA antigen and a peptide of any one of [1] to [7].

[22] The antigen-presenting cell of [21], induced by the method of [17].

[23] A vaccine for inhibiting proliferation of cells expressing genes of SEQ ID NO: 1, 3 and/or 5, wherein the vaccine comprises a peptide of any one of [1] to [7] as the active ingredient.

[24] The vaccine of [23], wherein the cell is a cancer cell.

[25] The vaccine of [24], wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangincellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC and soft tissue tumor.

[26] The vaccine of [23], formulated for administration to a subject whose HLA antigen is HLA-A24 or HLA-A2.

[27] A method of treating or preventing a disease associated with the over-expression of the genes of SEQ ID NO: 1, 3 and/or 5 in a subject comprising administering to said subject a vaccine comprising one or more peptide of any one of [1] to [7], an immunologically active fragment thereof, or a polynucleotide encoding said peptide or immunologically active fragment.

[28] The method of [27], wherein the disease is cancer.

[29] The method of [28], wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangincellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC and soft tissue tumor.

[0014] Alternatively, the present invention also relates to a method of inducing cytotoxic T cells comprising the step of contacting a T-cell with the antigen-presenting cell produced by the method of [19].

[0015] These ~~and other~~ objects and features of the invention will become more fully

Claims

[1] An isolated peptide having cytotoxic T cell inducibility, wherein said peptide derived from amino acid sequence of SEQ ID NO: 2, 4, or 6.

[2] An isolated peptide of less than about 15 amino acids selected from the group consisting of peptides comprising the amino acid sequences of SEQ ID NO: 7, 8 and 12, or a peptide having cytotoxic T cell inducibility, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 7, 8 and 12, wherein 1, 2, or several amino acids are substituted, deleted, or added.

[3] The peptide having cytotoxic T cell inducibility of claim 2, wherein the second amino acid from the N-terminus is phenylalanine, tyrosine, methionine, or tryptophan.

[4] The peptide having cytotoxic T cell inducibility of claim 2, wherein the C-terminal amino acid is phenylalanine, leucine, isoleucine, tryptophan, or methionine.

[5] An isolated peptide of less than about 15 amino acids selected from the group consisting of peptides comprising the amino acid sequences of SEQ ID NO: 9, 10, 11, 192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 253, 254 and 255, or a peptide having cytotoxic T cell inducibility, wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, 10, 11, 192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 253, 254 and 255, wherein 1, 2, or several amino acids are substituted, deleted, or added.

[6] The peptide having cytotoxic T cell inducibility of claim 5, wherein the second amino acid from the N-terminus is leucine or methionine.

[7] The peptide having cytotoxic T cell inducibility of claim 5, wherein the C-terminal amino acid is valine or leucine.

[8] A vector in which the DNA encodes peptides of any one of claims 1 to 7.

[9] A pharmaceutical composition for treating or preventing a disease associated with over-expression of the genes of SEQ ID NO: 1, 3 and/or 5, said composition comprising one or more peptides of claim 2 or 5.

[10] The pharmaceutical composition of claim 9, wherein the disease is cancer.

[11] The pharmaceutical composition of claim 10, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC and soft tissue tumor.

[12] An exosome that presents on its surface a complex comprising a peptide of claim

2 or 5 and an HLA antigen.

[13] The exosome of claim 12, wherein the HLA antigen is HLA-A24.

[14] The exosome of claim 13, wherein the HLA antigen is HLA-A2402.

[15] The exosome of claim 12, wherein the HLA antigen is HLA-A2.

[16] The exosome of claim 13, wherein the HLA antigen is HLA-A0201.

[17] A method of inducing antigen-presenting cells having a high cytotoxic T cell inducibility comprising the step of contacting an antigen-presenting cell with a peptide of claim 2 or 5.

[18] A method of inducing cytotoxic T cells by contacting a T cell with a peptide of claim 2 or 5.

[19] A method of inducing antigen-presenting cells having high cytotoxic T cell inducibility, said method comprising the step of transferring a gene comprising a polynucleotide encoding a peptide of claim 2 or 5 to an antigen-presenting cell.

[20] An isolated cytotoxic T cell, which is induced by contacting a T cell with a peptide of any one of claims 1 to 8 or which is transduced with the nucleic acids encoding the TCR subunits polypeptides binding with a peptide of any one claims 1 to 8 in the context of HLA-A24 or HLA-A2.

[21] An antigen-presenting cell, which comprises a complex formed between an HLA antigen and a peptide of claim 2 or 5.

[22] The antigen-presenting cell of claim 21, induced by the method of claim 17.

[23] A vaccine for inhibiting proliferation of cells expressing genes of SEQ ID NO: 1, 3 and/or 5, wherein the vaccine comprises a peptide of claim 2 or 5 as the active ingredient.

[24] The vaccine of claim 23, wherein the cell is a cancer cell.

[25] The vaccine of claim 24, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangincellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC and soft tissue tumor.

[26] The vaccine of claim 23, formulated for administration to a subject whose HLA antigen is HLA-A24 or HLA-A2.

[27] A method of treating or preventing a disease associated with the over-expression of the genes of SEQ ID NO: 1, 3 and/or 5 in a subject comprising administering to said subject a vaccine comprising one or more peptide of claim 2 or 5, an immunologically active fragment thereof, or a polynucleotide encoding said peptide or immunologically active fragment.

[28] The method of claim 27, wherein the disease is cancer.

[29] The method of claim 28, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangincellular carcinoma,

CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC and soft tissue tumor.

Claims

1. An isolated peptide having cytotoxic T cell inducibility, wherein said peptide is derived from the amino acid sequence of SEQ ID NO:4 or 6, wherein the peptide is less than 15 amino acids, wherein the peptide comprises the amino acid sequence selected from the group consisting of SEQ ID NOs:12, 192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 249, 253, 254 and 255.

2. An isolated peptide of less than 15 amino acids, wherein the peptide comprises the amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:12; and

(b) the amino acid sequence of SEQ ID NO:12, in which 1 or 2 amino acids are substituted, deleted, or added,

wherein the peptide has cytotoxic T cell inducibility.

3. The peptide of Claim 2, wherein the second amino acid from the N-terminus is phenylalanine, tyrosine, methionine, or tryptophan.

4. The peptide of Claim 2 or 3, wherein the C-terminal amino acid is phenylalanine, leucine, isoleucine, tryptophan, or methionine.

5. An isolated peptide of less than 15 amino acids, wherein the peptide comprises the amino acid sequence selected from the group consisting of:

(a) the amino acid sequence selected from the group consisting of: SEQ ID NOs:192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 249, 253, 254 and 255; and

(b) the amino acid sequence selected from the group consisting of SEQ ID NOs:192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 249, 244, 253, 254 and 255, in which 1 or 2 amino acids are substituted, deleted, or added,

wherein the peptide has cytotoxic T cell inducibility.

6. The peptide of Claim 5, wherein the second amino acid from the N-terminus is leucine or methionine.

7. The peptide of Claim 5 or 6, wherein the C-terminal amino acid is valine or leucine.

8. An isolated peptide consisting of the amino acid sequence selected from the group consisting of SEQ ID NOs:12, 192, 195, 197, 209, 225, 226, 228, 230, 240, 241, 243, 244, 249, 253, 254 and 255.

9. A vector comprising DNA which encodes the peptide of any one of Claims 1 to 8.

10. A pharmaceutical composition for treating or preventing a disease associated with over-expression of either or both of the genes of SEQ ID NO:3 and 5, said composition comprising one or more peptides of any one of Claims 1 to 8.

11. An exosome that presents on its surface a complex comprising a peptide of any one of Claims 1 to 8 and an HLA antigen.

12. The exosome of Claim 11, wherein the HLA antigen is HLA-A24 or HLA-A2.

13. The exosome of Claim 12, wherein the HLA antigen is HLA-A2402 or HLA-A0201.

14. An *in vitro* method of inducing antigen-presenting cells having cytotoxic T cell inducibility, said method comprising the step of:

(a) contacting an antigen-presenting cell with a peptide of any one of Claims 1 to 8; or

(b) transferring a gene comprising a polynucleotide encoding a peptide of any one of Claims 1 to 8 to an antigen-presenting cell.

15. An *in vitro* method of inducing cytotoxic T cells, said method comprising the step of contacting $CD8^+$ T cells with antigen-presenting cells induced by the method of Claim 14.

16. An isolated cytotoxic T cell, which is induced by the method of Claim 15 or which is transduced with the nucleic acids encoding the TCR subunits polypeptides binding with a peptide of any one of Claims 1 to 8 in the context of HLA-A24 or HLA-A2.

17. An isolated antigen-presenting cell, which comprises a complex formed between an HLA antigen and a peptide of any one of Claims 1 to 8.

18. The antigen-presenting cell of Claim 17, induced by the method of Claim 14.

19. A vaccine for inhibiting proliferation of cells expressing either or both of the genes of SEQ ID NO:3 and 5, wherein the vaccine comprises a peptide of any one of Claims 1 to 8 as an active ingredient.

20. The vaccine of Claim 19, wherein the cell is a cancer cell.

21. The vaccine of Claim 19 or 20, formulated for administration to a subject whose HLA antigen is HLA-A24 or HLA-A2.

22. The pharmaceutical composition of Claim 10, wherein the disease is cancer.

23. The pharmaceutical composition of Claim 22, or the vaccine of Claim 20, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, cholangiocellular carcinoma, CML, colorectal cancer, gastric cancer, NSCLC, lymphoma, osteosarcoma, prostate cancer, renal carcinoma, SCLC and soft tissue tumor.