CYTOTOXIC HEXIM1 PEPTIDES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore provisional application Nos. 10201501642V filed on 4 March 2015 and 10201504803T filed on 17 June 2015, the contents of which are hereby incorporated by reference in their entirety for all purposes.

FIELD OF THE INVENTION

[001] The present invention generally relates to the field of biochemistry. In particular, the present invention relates to cytotoxic peptides, compositions containing cytotoxic peptides and the use of the peptides and compositions in the prevention and treatment of cancers.

BACKGROUND OF THE INVENTION

[0002] Cancer is the second leading cause of death worldwide, accounting for 8.2 million deaths in 2012. It is expected that annual cancer cases will rise from 14 million in 2012 to 22 million within the next two decades.

[0003] Conventional treatments for cancer using radiation or chemotherapeutic drugs target rapidly proliferating cells and induce such cells to undergo apoptosis. Most chemotherapeutic agents exhibit some degree of toxicity toward normal cells at therapeutic doses, causing undesired side effects that may be dose limiting, thereby reducing the usefulness of the drugs. Furthermore, these traditional methods of treatment are not successful in treating many types of cancers, particularly those that are resistant to apoptotic stimuli. Since the cytotoxic agents in most chemotherapeutic drugs act primarily through p53-dependent induction of apoptosis, and p53 is often mutated in cancers cells, resistance to chemotherapy is often observed in cancer patients. As a consequence, there is an ongoing need for new therapeutic agents that are more effective in treating cancers.

SUMMARY

[0004] In one aspect, there is provided an isolated cytotoxic peptide conjugate comprising an isolated cytotoxic peptide conjugated to an internalizing peptide, or an internalizing protein, or an antibody, wherein the isolated cytotoxic peptide has a sequence identity of at

least 66% to SEQ ID NO: 3, and comprises an amino acid sequence corresponding to the functional part of the amino acid sequence represented by the consensus sequence Q_1 -L_2 -G_3 - X_4 -X_5 - X_6 -X_7 -X_8 -X_9 -R_{10} -P_{11} -S_{12} -X_{13} -X_{14} -X_{15} -R_{16} -H_{17} -W_{18} (SEQ ID NO: 36), wherein the functional part comprises X_8 -X_9 -R_{10} and X_{13} -X_{14} -X_{15} of SEQ ID NO: 36, and wherein i) X_8 and X_9 are independently K or R or H or A; and ii) X_{13}, X_{14} and X_{15} are independently K or R or H. In another aspect, there is provided an isolated cytotoxic peptide conjugate comprising an isolated cytotoxic peptide conjugated to a nanoparticle or a microparticle, wherein the isolated cytotoxic peptide has a sequence identity of at least 66% to SEQ ID NO: 3, and comprises an amino acid sequence corresponding to the functional part of the amino acid sequence represented by the consensus sequence Q_1 -L_2 -G_3 -X_4 -X_5 - X_6 -X_7 -X_8 -X_9 -R_{10} -P_{11} -S_{12} -X_{13} -X_{14} -X_{15} -R_{16} -H_{17} -W_{18} (SEQ ID NO: 36), wherein the functional part comprises X_8 -X_9 -R_{10} and X_{13} -X_{14} -X_{15}, and wherein i) X_8 and X_9 are independently K or R or H or A; and ii) X_{13}, X_{14} and X_{15} are independently K or R or H. In one example, the present disclosure provides an isolated cytotoxic peptide having sequence identity of at least 66% to SEQ ID NO: 3 or functional part thereof.

[0005] In another example, the present disclosure provides an isolated cytotoxic peptide comprising at least one amino acid sequence represented by the consensus sequence Q_1 -L_2 -G_3 -X_4 -X_5 -X_6 -X_7 -X_8 -X_9 -R_{10} -P_{11} -S_{12} -X_{13} -X_{14} -X_{15} -R_{16} -H_{17} -W_{18} (SEQ ID NO: 36), or functional part thereof, wherein X is any amino acid.

[0006] In one aspect, the present invention refers to an isolated nucleic acid molecule comprising the nucleotide sequence encoding the isolated cytotoxic peptide conjugate as described herein.

[0007] In another aspect, the present invention refers to a vector comprising the isolated nucleic acid molecule as described herein.

[0008] In another aspect, the present invention refers to a pharmaceutical composition comprising the isolated cytotoxic peptide conjugate, the isolated nucleic acid molecule, or the vector as described herein.

[0009] In yet another aspect, the present invention refers to use of the isolated cytotoxic peptide conjugate of the present invention, the isolated nucleic acid molecule of the present invention, the vector of the present invention or the pharmaceutical composition of the present invention in the manufacture of a medicament for treating or preventing cancer in a subject. In one example, there is provided a method of treating or preventing cancer in a subject, comprising administering the isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule, the vector or the pharmaceutical composition as described herein.

[0010] In one aspect, the present invention refers to the use of the isolated cytotoxic peptide conjugate of the present invention, the isolated nucleic acid molecule of the present invention, the vector of the present invention or the pharmaceutical composition of the present invention in the manufacture of a medicament for eliminating undifferentiated stem cells in a subject having stem cell transplantation. In one example, there is provided a method of eliminating undifferentiated stem cells in a subject having stem cell transplantation, comprising administering the isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule, the vector or the pharmaceutical composition as described herein.

[0011] In another aspect, the present invention refers to the use of the isolated cytotoxic peptide conjugate of the present invention, the isolated nucleic acid molecule of the present invention, the vector of the present invention or the pharmaceutical composition of the

present invention in the manufacture of a medicament for treating or preventing obesity in a subject. In one example, there is provided a method of treating or preventing obesity in a subject, comprising administering the isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule, the vector or the pharmaceutical composition as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0013] **Fig. 1** shows FGF-tagged BR peptide (SEQ ID NO: 19) and FGF-tagged mutant BR peptides induce cell death in various cell lines. FGF tag is a cell membrane-translocating

Claims

1. An isolated cytotoxic peptide conjugate comprising an isolated cytotoxic peptide conjugated to an internalizing peptide, or an internalizing protein, or an antibody,
wherein the isolated cytotoxic peptide has a sequence identity of at least 66% to SEQ ID NO: 3, and comprises an amino acid sequence corresponding to the functional part of the amino acid sequence represented by the consensus sequence Q_1-L_2-G_3-X_4-X_5-X_6-X_7-X_8-X_9-R_{10}-P_{11}-S_{12}-X_{13}-X_{14}-X_{15}-R_{16}-H_{17}-W_{18} (SEQ ID NO: 36),
wherein the functional part comprises X_8-X_9-R_{10} and X_{13}-X_{14}-X_{15} of SEQ ID NO: 36, and wherein
i) X_8 and X_9 are independently K or R or H or A; and
ii) X_{13}, X_{14} and X_{15} are independently K or R or H.

2. An isolated cytotoxic peptide conjugate comprising an isolated cytotoxic peptide conjugated to a nanoparticle or a microparticle,
wherein the isolated cytotoxic peptide has a sequence identity of at least 66% to SEQ ID NO: 3, and comprises an amino acid sequence corresponding to the functional part of the amino acid sequence represented by the consensus sequence Q_1-L_2-G_3-X_4-X_5-X_6-X_7-X_8-X_9-R_{10}-P_{11}-S_{12}-X_{13}-X_{14}-X_{15}-R_{16}-H_{17}-W_{18} (SEQ ID NO: 36),
wherein the functional part comprises X_8-X_9-R_{10} and X_{13}-X_{14}-X_{15}, and wherein
i) X_8 and X_9 are independently K or R or H or A; and
ii) X_{13}, X_{14} and X_{15} are independently K or R or H.

3. The isolated cytotoxic peptide conjugate of claim 1 or 2, wherein
i) X_8 and X_9 are the same, and are K or R or H or A; and
ii) X_{13}, X_{14} and X_{15} are the same, and are K or R or H.

4. The isolated cytotoxic peptide conjugate of any one of claims 1 to 3, wherein the amino acid sequence is selected from the group consisting of: i) QLGKKKHRRRPSKKKRHW (SEQ ID NO: 3), ii) QLGRRRHRRRPSRRRRHW (SEQ ID NO: 4), iii) QLGKKILAARPSKKKRHW (SEQ ID NO: 5), iv)

HRRRPSKKKRHW (SEQ ID NO: 37) and v) QLGAAAHRRRPSKKKRHW (SEQ ID NO: 38).

5. The isolated cytotoxic peptide conjugate of any one of claims 1, 3 and 4, wherein the internalizing peptide or internalizing protein is a cell-penetrating peptide or protein, and/or a cell-targeting peptide or protein.

6. The isolated cytotoxic peptide conjugate of claim 5, wherein the cell-penetrating peptide is selected from the group consisting of transactivator of transcription (TAT), penetratin, R6-Pen, transportan, MPG peptide, sweet arrow peptide (SAP), peptide from vascular endothelial-cadherin protein (pVEC), Pep-1 (KETWWETWWTEWSQPKKKRKV) (SEQ ID NO: 6), polylysines, polyarginines, model amphipathic peptide (MAP) and R6W3 (RRWWRRWRR) (SEQ ID NO: 7).

7. The isolated cytotoxic peptide conjugate of claim 5, wherein the cell-targeting peptide or protein is a cancer cell-targeting peptide or protein.

8. The isolated cytotoxic peptide conjugate of claim 7, wherein the cancer cell-targeting peptide or protein is selected from the group consisting of arginine-glycine-aspartic acid (RGD), asparagine-glycine-arginine (NGR), TCP-1 phage peptide (TCP-1) and anyone of the peptides of SEQ ID Nos: 8 – 11, 64 – 789 and 795 – 798.

9. The isolated cytotoxic peptide conjugate of claim 5, wherein the cell-targeting peptide or protein is a lipocyte targeting peptide or protein.

10. The isolated cytotoxic peptide conjugate of claim 9, wherein the lipocyte targeting peptide is a CKGGRAKDC peptide (SEQ ID NO: 39).

11. The isolated cytotoxic peptide conjugate of claim 5, wherein the cell-targeting peptide or protein is a stem cell targeting peptide or protein.

12. The isolated cytotoxic peptide conjugate of claim 11, wherein the stem cell targeting peptide or protein is selected from the group consisting of an embryonic stem cell targeting peptide or protein, an induced pluripotent stem cell targeting peptide or protein and a tissue stem cell targeting peptide or protein.

13. The isolated cytotoxic peptide conjugate of claim 12, wherein the embryonic stem cell targeting peptide is selected from the group consisting of the sequences of SEQ ID NO: 41 – 63.

14. The isolated cytotoxic peptide conjugate of any one of claims 1 and 3 to 13, wherein the antibody is an antibody for cancer therapy.

15. The isolated cytotoxic peptide conjugate of claim 14, wherein the antibody for cancer therapy is selected from the group consisting of Ado-trastuzumab emtansine (CAS No. 1018448-65-1), Alemtuzumab (CAS No. 216503-57-0), Bevacizumab (CAS No. 216974-75-3), Blinatumomab (CAS No. 853426-35-4), Brentuximab vedotin (CAS No. 914088-09-8), Cetuximab (CAS No. 205923-56-4), Daratumumab (CAS No. 945721-28-8), Denileukin diftitox (CAS No. 173146-27-5), Gemtuzumab (CAS No. 220578-59-6), Ibritumomab tiuxetan (CAS No. 174722-31-7), Ipilimumab (CAS No. 477202-00-9), Nivolumab (CAS No. 946414-94-4), Ofatumumab (CAS No. 679818-59-8), Panitumumab (CAS No. 339177-26-3), Rituximab (CAS No. 174722-31-7), Tositumomab (CAS No. 192391-48-3) and Trastuzumab (CAS No. 180288-69-1).

16. The isolated cytotoxic peptide conjugate of any one of claims 2 to 4, wherein the nanoparticle or microparticle is made from a material selected from the group consisting of metal, silica, carbon, polymeric materials, and mixtures thereof.

17. The isolated cytotoxic peptide conjugate of any one of claims 1 to 16, wherein the peptide has a modification selected from the group consisting of glycosylation, sulfation, phosphorylation, ubiquitination, methylation, lipidation, biotinylation, hydroxylation and acetylation.

18. An isolated nucleic acid molecule comprising the nucleotide sequence encoding the isolated cytotoxic peptide conjugate of any one of claims 1, 3 to 15, and 17.

19. A vector comprising the isolated nucleic acid molecule of claim 18.

20. A pharmaceutical composition comprising the isolated cytotoxic peptide conjugate of any one of claims 1-17, the isolated nucleic acid molecule of claim 18 or the vector of claim 19.

21. Use of the isolated cytotoxic peptide conjugate of any one of claims 1-17, the isolated nucleic acid molecule of claim 18, the vector of claim 19 or the pharmaceutical composition of claim 20 in the manufacture of a medicament for treating or preventing cancer in a subject.

22. The use of claim 21, wherein the cancer is selected from the group consisting of breast cancer, colorectal cancer, gastric cancer, melanoma, pancreatic cancer, skin cancer, leukemia, myeloma, hepatocellular cancer, pancreatic cancer, cervical cancer, ewings tumour, neuroblastoma, kaposis sarcoma, prostate cancer, bladder cancer, melanoma, lung cancer - non small cell lung cancer (NSCLC), and small cell lung cancer (SCLC), head and neck cancer, renal cancer, lymphoma, prostate cancer, neuroblastoma, a blood cancer, testicular cancer, ovarian cancer, liver cancer or esophageal cancer, cervical cancer, non-melanoma skin cancer, glioblastoma, carcinoma, uterus cancer, chronic lymphoid leukemia, lymphoblastic leukemia, follicular lymphomas, melanomas, malignant homeopathies,acute leukemia, basal cell carcinoma, bone cancer, brain and central nervous system (CNS) cancer, connective tissue cancer, eye cancer, kidney cancer, larynx cancer, Hodgkin's lymphoma, non- Hodgkin's lymphoma, oral cavity (lip, tongue, mouth, and pharynx) cancer, and rhabdomyosarcoma.

23. The use of claim 22, wherein the cancer is breast cancer.

24. Use of the isolated cytotoxic peptide conjugate of any one of claims 1-17, the isolated nucleic acid molecule of claim 18, the vector of claim 19 or the pharmaceutical composition of claim 20 in the manufacture of a medicament for eliminating undifferentiated stem cells in a subject having stem cell transplantation.

25. The use of claim 24, wherein the stem cell transplantation is selected from the group consisting of: embryonic stem cells transplantation, induced pluripotent stem cells transplantation, tissue stem cells transplantation, and a mixture thereof.

26. Use of the isolated cytotoxic peptide conjugate of any one of claims 1-17, the isolated nucleic acid molecule of claim 18, the vector of claim 19 or the pharmaceutical composition of claim 20 in the manufacture of a medicament for treating or preventing obesity in a subject.