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

peptide derived from Kaposi fibroblast growth factor. **(A)** Alignment of the HDM2 ubiquitination sites of p53 (amino acids (AAs) 370-386, SEQ ID NO: 34) and HEXIM1 (AAs 150-161, SEQ ID NO: 35) shows that ubiquitin sites of p53 and HEXIM1 exhibit similar distribution of the lysine residues (the HDM2-ubiquitinated lysine residues are underlined). **(B)** Alignment of wild-type and mutant HEXIM1 BR peptides (RRR12 and ILAA) indicates the amino acid residues that have been mutated (the mutated residues are underlined). **(C)** Cell viability plots showing the effects of FGF-tagged BR peptide in various cancer cell lines. Cancer cell lines: Acute Myeloid Leukemia cell lines -- AML2, AML3, cervical cancer cell line – HeLa, and non-cancer cell lines -- Human Embryonic Kidney 293 (293) and Human Foreskin Fibroblast (HFF) cell lines were treated overnight with various concentrations of indicated FGF-fused peptides before cell viability was determined by Cell-Titer Glo assays. Cells treated with the FGF-X13 peptide (SEQ ID NO: 18) were used as a negative control. The results show that FGF-tagged BR peptide induces cell death in AML2, AML3, HeLa, HEK293 and HFF cell lines. **(D)** Cell viability plots showing the effects of FGF-tagged BR peptide and variants thereof in human colon cancer cell line HCT116 with wild-type p53 (p53 WT) or p53 knockout (p53KO). HCT116 (p53 WT) and HCT116 (p53 KO) cells were treated with FGF-X13, FGF-BR, FGF-BR-RRR12 (SEQ ID NO: 20) and FGF-BR-ILAA (SEQ ID NO: 21) peptides at various concentrations overnight before cell viability assays were performed. Data representative of at least three independent experiments performed in triplicate were shown with values expressed as mean ± SD. The results indicate that FGF-tagged BR peptide shows similar level of cytotoxicity in HCT116 p53 wild-type (p53 WT) and HCT116 p53 knockout (p53 KO) cell lines. FGF-tagged mutant BR peptides, BR(RRR12) and BR(ILAA) also show cytotoxicity in both HCT116 (p53 WT) and HCT116 (p53KO) cell lines.

[0014] **Fig. 2** shows FGF-/LTV-tagged BR peptide induced rapid cell death independently of apoptosis. **(A)** shows representative time-lapse differential interference contrast (DIC) snapshots of treated HCT116 (p53 WT) and HCT116 (p53 KO) cells (bar represents100 μm). HCT116 (p53 WT) and HCT116 (p53 KO) cells were cultured on glass slides overnight, treated with vehicle control (0.5% dimethyl sulfoxide (DMSO)) or the indicated FGF peptides (30 μM). Time-lapse DIC imaging highlights dynamic morphological changes in treated HCT116 cells by spinning disk confocal microscopy. The results indicate that in both HCT116 (p53 WT) and HCT116 (p53KO) cell lines, FGF tag alone does not affect

morphology or viability of the cells. In contrast, FGF-BR peptide rapidly induces drastic changes to the cell morphology with rupturing of the plasma membrane accompanied with damages to the nuclear membrane and abnormalities to the nucleolus in both cell types. **(B)** cytotoxicity fluorescence plots of breast cancer cells MCF7 showing the effect of pan-caspase inhibitor z-VAD-Fmk on cells treated with LTV-tagged BR peptide. LTV is a breast cancer cell targeting peptide. MCF7 cells were incubated with or without z-VAD-Fmk (for 90 min) and then with indicated LTV-tagged peptides (30 μM) for thirty minutes at 37°C. Cells treated with LTV-X13 peptide (SEQ ID NO: 14) or vehicles, DMSO (0.5%), were used as negative controls. Treated cells were subjected to the cytotoxicity assay as described in Example 9 – Materials and Methods. Data representative of at least three independent experiments performed in triplicate were shown with values expressed as mean ± SD. The results indicate that LTV-tagged BR (SEQ ID NO: 15) has significant cytotoxic effect on MCF7 cell line, and the effect could not be inhibited by z-VAD-Fmk.

[0015] **Fig. 3** shows FGF-tagged BR peptide decreases mitochondrial membrane potential in HCT116 (p53 WT) cell line but not in HCT116 (p53 KO) cell line. (A) shows bar chart of mitochondrial membrane potential in HCT116 (p53 WT) cell line treated with negative and positive controls and the FGF-tagged BR peptide. (B) shows bar chart of mitochondrial membrane potential in HCT116 (p53 KO) cell line treated with negative and positive controls and the FGF-tagged BR peptide. HCT116 (p53 WT) and HCT116 (p53 KO) cells were cultured in 96-well plates, treated with indicated FGF-tagged peptides (30 μM) for three minutes. A mitochondrial membrane depolarizer, carbonyl cyanide 3-chlorophenylhydrazone (CCCP), was used as a positive control. Cells treated with FGF-X13 peptide or vehicle, DMSO (0.5%), was used as negative controls. Treated cells were subjected to mitochondrial membrane potential (MMP) measurement using JC-1 fluorescence-based assay for six independent experiments. Results were summarized as mean ± SD (***, $P < 0.0001$; ns: not significant, Student's t test). The results indicate that FGF-tagged BR peptide decreased mitochondrial membrane potential in HCT116 cell line is dependent on p53.

[0016] **Fig. 4** shows FGF-tagged BR peptide alters the sub-cellular localization and protein level of endogenous NPM. **(A)** representative immunofluorescence images showing the sub-cellular localization of α-NPM (bar represents 10μm). HCT116 (p53 WT) and HCT116 (p53KO) cells were cultured on glass slides overnight, treated with FGF-X13 or FGF-BR (30 μM). Cells treated with FGF-X13 peptide or vehicle, DMSO (0.5%), was used

as controls. Treated cells were immunostained with an anti-NPM antibody and analyzed by laser scanning confocal microscopy (Zeiss). Nuclei were visualized by staining with 4',6-diamidino-2-phenylindole (DAPI). The results indicate that NPM is mislocated into the cytoplasm in cells treated FGF-tagged BR peptide, in both HCT116 (p53 WT) and HCT116 (p53 KO) cell lines. **(B)** results of western blot showing the expression level of α-NPM protein (α-actin was used as the internal control. HCT116 cells were plated overnight prior to addition of vehicle control (0.5% DMSO) or indicated peptides (30 μM) at 37°C. Lysates were subsequently harvested and subjected to western blotting with anti-NPM and anti-actin antibodies. The level of NPM protein was quantified as described in Example 9 – Materials and Methods. The results show that endogenous level of NPM is reduced in cells treated with FGF-tagged BR peptide, in both HCT116 (p53 WT) and HCT116 (p53 KO) cell lines.

[0017] **Fig. 5 C**ell viability plots showing the effects of LTV-tagged BR peptide and variants thereof, as well as untagged BR peptide and variants thereof, in two breast cancer cell lines, **(A)** MCF7 and **(B)** MDA-MB-231. Cells were treated with indicated LTV-tagged peptides at different concentrations overnight before cell viability assays were performed. Both LTV-tagged BR and LTV-tagged BR (RRR12) peptides decreased cell viability in (A) MCF7 and (B) MDA-MB-231 cell lines. Effects of the indicated un-tagged peptides on the viability of **(C)** MCF7 and **(D)** MDA-MB-231 cells were analyzed by cell viability assays. The untagged X13 was used as a negative control. The results indicate that untagged BR and BR (RRR12) peptides have no effect on cell viability in (C) MCF7 and (D) MDA-MB-231 cell lines. Data representative of at least three independent experiments performed in triplicate were shown with values expressed as mean ± SD. The results indicate that LTV-tagged BR peptide decreases cell viability in breast cancer cells, while BR peptide alone shows no effect on cell viability.

[0018] **Fig. 6** shows the untagged HEXIM1 BR peptide fails to enter cells by itself, while another cytotoxic peptide, KLA, can enter the cell without a cell penetrating and/or cell targeting tag. **(A)** shows images of confocal microscopy of MCF7 cells treated with vehicle control and the indicated peptides (bar represents 10 μm). MCF7 cells were cultured on glass slides overnight before incubation with the indicated FITC-labeled peptides (30 μM) for 30 min and subsequently analyzed by laser scanning confocal microscopy. Nuclei were visualized by DAPI. Cells treated with vehicle, DMSO (0.5%) were used as control. Pointed arrow indicates FITC fluorescence. The results show that no FITC fluorescence can be

defected in cells treated with BR-FITC peptide, while FITC fluorescence can be detected in cells treated with LTC-BR-FITC, KLA-FITC and LTV-KLA-FITC peptides. **(B)** shows bar chart of FITC-positive cells quantified by flow cytometry. Data represent percentage of fluorescence-positive cells in total cell population. Results were summarized as mean ± SD of three independent experiments (***, $P < 0.0001$, Student's *t* test). The results indicate that no significant cellular uptake of BR-FITC was observed, while the cellular uptake of KLA-FITC, LTV-BR-FITC and LTV-KLA-FITC is significantly higher compared to BR-FITC.

[0019] **Fig. 7** shows cell viability plots (upper panel) of cells treated control, LTV-tagged BR and mutated variants thereof (sequences indicated in the lower panel). MDA-MB-231 breast cancer cells were treated with indicated truncated LTV-tagged peptides at various concentrations overnight before cell viability assays were performed. Cells treated with LTV-X13 were used as negative control, cells treated with LTV-BR were used as positive control. Data representative of at least three independent experiments performed in triplicate were shown with values expressed as mean ± SD. The results indicate that LTV-tagged BR peptide requires all three stretches of basic residues (KKK, RRR and KKK) to establish full potency in cell killing.

[0020] **Fig. 8** shows cell viability plots (upper panel) of cells treated control, LTV-tagged BR and alanine substituted variants thereof (sequences indicated in the lower panel). MDA-MB-231 breast cancer cells were treated with indicated alanine-substituted LTV-tagged peptides at 10 μM and 30 μM overnight before cell viability assays were performed. Cells treated with LTV-X13 were used as negative control, cells treated with LTV-BR were used as positive control. Data representative of at least three independent experiments performed in triplicate were shown with values expressed as mean± SD. The results indicate that alanine residue substitution in the middle and C-terminal regions of the HEXIM1 BR peptide abolished the cytotoxic activity of LTV-BR.

[0021] **Fig. 9** shows cell viability plots of various cell lines treated with control and LTV-tagged BR and LTV-tagged KLA peptides. Chinese hamster ovary cancer cell line, CHO-K1; multiple myeloma cell line, OPM-2, human foreskin fibroblasts, HFF; and human lung fibroblasts, WI-38 were treated with indicated LTV-fused peptides at various concentrations overnight before cell viability assays were performed. Cells treated with LTV-X13 was used as a negative control. Data representative of at least three independent experiments performed in triplicate were shown with values expressed as mean ± SD. The results indicate that LTV-

tagged KLA peptide exhibits non-specific killing on non-breast cancer cell lines and normal human fibroblasts, while LTV-tagged BR peptide does not show such non-specific cytotoxicity.

[0022] **Fig. 10** shows cell viability plots of (A) undifferentiated 3T3 cells and (B) adipose cells treated with BR_D and KLA_D peptides tagged with lipocyte-targeting peptide, ATS (ATS-BR_D and ATS-KLA_D peptides). The results indicate that the ATS-BR_D peptide has no cytotoxic effect on undifferentiated 3T3 mouse fibroblast cells, but has significant cytotoxic effect on differentiated adipose cells. The cytotoxic effect of ATS-BR_D peptide on adipose cells is of similar potency as ATS-KLA_D peptide.

[0023] **Fig. 11** shows that the cytotoxic effect exerted by BR is independent of p53. AML2 and AML3 cells were treated with FGF-tagged p53 and FGF-tagged BR peptides at the indicated concentrations. Cell viability was measured 16 hours post-incubation by Cell-Titer Glo. Cells treated with vehicle, DMSO (0.5%), were used as control. (A) Cell viability plots showing the effects of FGF-tagged p53 and FGF-tagged BR peptides in AML2 cell line. (B) Cell viability plots showing the effects of FGF-tagged p53 and FGF-tagged BR peptides in AML3 cell line.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

[0024] In the search for new therapeutic agents that are more effective in treating cancers, it was found that the basic region (BR) of hexamethylene bisacetamide inducible protein 1 (HEXIM1, represented by SEQ ID NO: 1) exerts its cytotoxic effects independent of the status of p53. Thus, the present disclosure provides an isolated peptide that has the cytotoxic activity of the BR of HEXIM1.

[0025] In one example, there is provided an isolated cytotoxic peptide having sequence identity of at least 66% to SEQ ID NO: 3, or functional part thereof. In some examples, the isolated cytotoxic peptide has sequence identity of at least 72%, at least 77%, at least 83%, at least 88% or at least 94%to SEQ ID NO: 3, or functional part thereof. In some other examples, the isolated cytotoxic peptide has sequence identity of 66% to 78%, 77% to 89%, 88% to 95% to SEQ ID NO: 3, or functional part thereof.

[0026] As used herein, the basic region (BR) of HEXIM refers to the region between AAs 150-180 of HEXIM1, which includes a stretch of basic residues. The BR is represented by the sequence of KKKHRRRPSKKKRHWKPYYKLTWEEKKKFDE (SEQ ID NO: 2).

[0027] As used herein, an "isolated" peptide is intended to mean a peptide removed from its native environment, such as recombinantly produced peptides expressed in host cells and native or recombinant peptides which have been substantially purified by any suitable technique. Isolated peptides according to the present disclosure further include such compounds produced synthetically.

[0028] The terms “peptide” used herein refers to polymers of amino acid residues of any length. The polymer may be linear or branched, it may comprise modified amino acids or amino acid analogues, and it may be interrupted by chemical moieties other than amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention, for example disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labelling or bioactive component. The term peptide encompasses two or more naturally occurring or synthetic amino acids linked by a covalent bond (for example, an amide bond). The peptide as disclosed herein can be of various lengths, such as having 6 to 200, 8 to 190, 10 to 180, 12 to 170, 14 to 160, 16 to 150, 18 to 140, 20 to 130, 22 to 120, 24 to 110, 26 to 100, 28 to 90, 30 to 80, 32 to 70, 34 to 60, 36 to 50 or 38 to 45 amino acid residues.

[0029] As used herein, the term “amino acid” is defined as having at least one primary, secondary, tertiary or quaternary amino group, and at least one acid group, wherein the acid group may be a carboxylic, sulfonic, or phosphoric acid, or mixtures thereof. The amino groups may be “alpha”, “beta”, “gamma” to “omega” with respect to the acid group(s). Suitable amino acids include, without limitation, both the D- and L-isomers of the 20 common naturally occurring amino acids found in peptides (for example, A, R, N, C, D, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, V (as known by the one-letter or three letter abbreviations)) as well as the naturally occurring and unnaturally occurring amino acids prepared by organic synthesis or other metabolic routes.

[0030] The term “sequence identity” as used herein refers to a relationship between two or more peptide sequences, namely a reference sequence and a given sequence to be compared with the reference sequence. Sequence identity is determined by comparing the given sequence to the reference sequence after the sequences have been optimally aligned to

produce the highest degree of sequence similarity, as determined by the match between strings of such sequences. Upon such alignment, sequence identity is ascertained on a position-by-position basis, for example, the sequences are "identical" at a particular position if at that position, the amino acid residues are identical. The total number of such position identities is then divided by the total number of amino acid residues in the reference sequence to give % sequence identity. Sequence identity can be readily calculated by known methods. Some methods used to determine the sequence identity are designed to give the largest match between the sequences tested. Methods to determine sequence identity are codified in publicly available computer programs that determine sequence identity between given sequences. These programs optimally align sequences using default gap weights in order to produce the highest level of sequence identity between the given and reference sequences. As an illustration, by a peptide or polypeptide having a given amino acid sequence having at least, for example, 66% sequence identity to a reference amino acid sequence, it is intended that the given amino acid sequence of the peptide or polypeptide is identical to the reference sequence except that the given peptide or polypeptide sequence may include up to 34 amino acid alterations per each 100 amino acids of the reference amino acid sequence. In other words, to obtain a given peptide or polypeptide sequence having at least 66% sequence identity with a reference amino acid sequence, up to 34% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 34% of the total number of amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or the carboxyl terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in the one or more contiguous groups within the reference sequence. In some examples, residue positions that are not identical differ by conservative amino acid substitutions. However, conservative substitutions are not included as a match when determining sequence identity. For example, the isolated cytotoxic peptide QLGKKILAARPSKKKRHW (SEQ ID NO: 5) has 77.8% sequence identity with SEQ ID NO: 3; the isolated cytotoxic peptides QLGRRRHRRRPSKKKRHW (SEQ ID NO: 4) and HRRRPSKKKRHW (SEQ ID NO: 37) both have 66.7% sequence identity with SEQ ID NO: 3, the isolated cytotoxic peptide QLGAAAHRRRPSKKKRHW (SEQ ID NO: 38) has 83.3% sequence identity with SEQ ID NO: 3.

[0031] Thus, in some examples, the isolated cytotoxic peptide as described herein has at least 12, at least 13, at least 14, at least 15, at least 16 or at least 17amino acid residues that are the same as SEQ ID NO: 3, or functional part thereof. In some other examples, the isolated cytotoxic peptide has 12 to 14 or 15 to 17 amino acid residues that are the same as SEQ ID NO: 3, or functional part thereof.

[0032] The terms "cytotoxic" and "cytotoxicity" as used herein refer to the effect of a compound or composition of being toxic to the cells. Effects of cytotoxicity on cells include, but are not limited to, cell swelling, cell lysis, loss of cell membrane integrity, decreased rate of metabolism, decreased cell viability, cell deaths (by inducing apoptosis or necrosis). Thus, the cytotoxic effects can be exerted via various pathways. Examples of possible pathways include, but are not limited to, apoptosis-independent pathways, positive transcription elongation factor b (P-TEFb)-independent pathway, p53-independent pathway. The terms "low toxicity" or "reduced toxicity" as used in the present disclosure refer to a decrease in toxicity towards non-cancerous cells and tissue relative or compared to cancerous cells and tissue. In some examples, the low or reduced toxicity towards non-cancerous cells and tissue is at most about 0.9 times, at most about 0.8 times, at most about 0.7 times, at most about 0.6 times, at most about 0.5 times, at most about 0.4 times, at most about 0.3 times, at most about 0.2 times, at most about 0.1 times, or at most about 0.05 times the toxicity towards cancer cells and tissue samples obtained from the same or a different subject.

[0033] The term "apoptosis" as used herein refers to programmed cell death, characterized by cell changes such as blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and global mRNA decay.

[0034] As used herein, the term "functional part" of a peptide or amino acid sequence refers to the part of the peptide or the amino acid sequence that produces the overall function of a peptide or amino acid sequence. In some examples, the functional part of a peptide or amino acid sequence comprises 6 to 17, 7 to 16, 8 to 15, 9 to 14, 10 to 13 or 11 to 12amino acid residues of the peptide or amino acid sequence. In one example, the functional part of SEQ ID NO: 3 refers to the part of SEQ ID NO: 3 that has cytotoxic effect. In some specific examples, the functional part of SEQ ID NO: 3 comprises at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, at least thirteen, at least fourteen, at least fifteen, at least sixteen or at least seventeen amino acid residues of the peptide or amino acid sequence. In some other examples, the functional part of SEQ ID NO:

3 comprises at least the following stretches of amino acid residues RRR (AAs 8-10 of SEQ ID NO: 3) and KKK (AAs 13-15 of SEQ ID NO: 3). Thus, some examples of the isolated cytotoxic peptide as described herein comprises amino acid residues at positions corresponding to amino acids 8 to 10 and 13 to 15 of SEQ ID NO: 3. Exemplary peptides include, but are not limited to, the sequences of:

i) QLGKKKHRRRPSKKKRHW (SEQ ID NO: 3);

ii) HRRRPSKKKRHW (SEQ ID NO: 37); and

ii) QLGAAAHRRRPSKKKRHW (SEQ ID NO: 38).

[0035] The isolated cytotoxic peptide as described above may comprise at least one amino acid sequence that can be represented by a consensus sequence. Thus, in one example, there is provided an isolated cytotoxic peptide having sequence identity of at least 66% to SEQ ID NO: 3, or functional part thereof, 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. In some examples, the isolated cytotoxic peptide has sequence identity of at least 72%, at least 77%, at least 83%, at least 88% or at least 94%to SEQ ID NO: 3, or functional part thereof. In some other examples, the isolated cytotoxic peptide has sequence identity of 66% to 78%, 77% to 89%, 88% to 95% to SEQ ID NO: 3, or functional part thereof.

[0036] In another example, there is provided 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.

[0037] The term “consensus sequence” as used herein refers to an amino acid sequence determined by aligning a series of multiple sequences. It defines an idealized sequence that represents the predominant choice of amino acid at each corresponding position of the multiple sequences.

[0038] In some examples, X_4, X_5 and X_6 can be any amino acids or may be deleted. In some examples, X_4, X_5 and X_6 are positively charged amino acids. In some examples, X_4, X_5 and X_6 are the same. In some other examples, at least two of X_4, X_5 and X_6 are different from each other. In some examples, X_7 can be histidine (H) or leucine (L). In some examples, X_8 and X_9 are positively charged amino acids. In some other examples, X_8 and X_9 can be small amino acid residues including but are not limited to alanine (A). In some examples, X_8 and

X_9 are the same, while in some other examples, X_8 and X_9 are different. In some examples, X_{13}, X_{14} and X_{15} are positively charged amino acids. In some examples, X_{13}, X_{14} and X_{15} are the same. In some other examples, at least two of X_{13}, X_{14} and X_{15} are different from each other.

[0039] In general, the positively charged amino acids may include, but are not limited to, lysine (K), arginine (R) or histidine (H). The positively charged amino acids may also include positively charged unnatural amino acids such as ornithine; 2,4-diaminobutanoic acid 2,3-diaminopropanoic acid), 3-(aminoiminomethyl)amino-alanine, 2-amino-4-(aminoiminomethyl)aminobutanoic acid, N^6-(aminoiminomethyly) lysine, 2-amino-7-(aminoiminomethyl)aminoheptanoic acid, 2,7-diaminoheptanoic acid, 2, 8-diaminooxtanoic acid, 2, 9-diaminononanoic acid, 2,10-diaminodecanoic acid, 4-(aminoiminomethyl)phenylalanine or 4-(aminoiminomethyl)aminophenylalanine.

[0040] Exemplary peptides 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} include, but are not limited to, the following sequences:

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).

[0041] In one example, the isolated cytotoxic peptide as described herein excludes the sequence of QLGKKKHRRRPSKKKRHW (SEQ ID NO: 3).

[0042] In some examples, the isolated cytotoxic peptide comprising the amino acid sequence represented by the consensus sequence as described above can include one or more additional amino acids at either end of the amino acid sequence represented by the consensus sequence. For example, the isolated cytotoxic peptide can include at least 1, at least 3, at least 5, at least 7, at least 9, at least 11, at least 13, at least 15, at least 17, at least 19, or 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 additional amino acid residues at the N-terminal and/or the C-terminal of the amino acid sequence represented by the consensus sequence.

[0043] In some examples, the functional part of the amino acid sequence represented by the consensus sequence comprises at least the following stretches of amino acid residues X_8-

X_9-R_{10} and $X_{13}X_{14}X_{15}$. Exemplary peptides comprising such functional part include, but are not limited to, the following sequences:

i) HRRRPSKKKRHW (SEQ ID NO: 37); and

ii) QLGAAAHRRRPSKKKRHW (SEQ ID NO: 38).

[0044] In some other examples, the isolated cytotoxic peptide comprising the amino acid sequence represented by the consensus sequence as described above can include more than one amino acid sequence represented by the consensus sequence. In some specific examples, the isolated cytotoxic peptide as described above includes at least 2, at least 3 or at least 4 amino acid sequences represented by the consensus sequence. In some examples, at least 2 or at least 3 of the amino acid sequences represented by the consensus sequence are of the same sequence. In some other examples, all the amino acid sequences represented by the consensus sequence are different. In some examples, the more than one amino acid sequences represented by the consensus sequence are separated by 1 to 3, 4 to 6, 7 to 9, 10 to 12, 11 to 13, 14 to 16, 17 to 19, 20 to 22 or 23 to 25 other amino acid residues. In some other examples, at least 2 or at least 3 of the more than one amino acid sequences represented by the consensus sequence are not separated by any other amino acid residues.

[0045] In some examples, the isolated cytotoxic peptide as described herein can exert it cytotoxicity via the apoptosis-independent pathway, the p53-independent pathway or the p-TEFb-independent pathway. Thus, in some examples, the isolated cytotoxic peptide or conjugate thereof is effective against cancer resistant to p53-directed therapy, cancer resistant to the TEFb-directed pathway, or cancer resistant to therapies targeting other apoptosis-dependent pathways.

[0046] The term “resistant” or grammatical variants are used herein in the context of being drug-resistant, and refer to the reduction in effectiveness of a drug in curing a disease or condition. Drug resistance can result from, for example, defects in a pathway or defects in element(s) in a pathway targeted by the drug. In one specific example, a patient with a mutated or an otherwise defective p53 protein will show resistance to a drug targeting the p53-dependent pathway.

[0047] The isolated cytotoxic peptide as disclosed herein may contain peptide modifications. The term “modification” is used herein to refer to post-translational modification in general, which are modifications that occur on a peptide usually after its translation by ribosome is complete. Post-translational modification generally refers to the

addition of a functional group covalently to a protein. These modifications include, but are not limited to, phosphorylation, glycosylation, sulfation, biotinylation, hydroxylation, acetylation, ubiquitination, nitrosylation, methylation, acetylation and lipidation.

[0048] In some examples, the isolated cytotoxic peptide as described herein contains at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, or 1 to 4, 5 to 8, 9 to 12, 13 to 16, 17 to 20, 21 to 24, 25 to 28, 29 to 32, 33 to 36, 37 to 40, 41 to 44, 45 to 48 or 49 to 52 modified amino acid residues. In some examples, all the modified amino acid residues contain the same type of modification. In some other examples, at least some of the modified amino acid residues contain different types of modifications. In some examples, wherein the isolated cytotoxic peptide comprises more than 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), at least two of the amino acid sequences represented by the consensus sequence contain the same amino acid modifications. In some other examples, wherein the isolated cytotoxic peptide comprises more than one amino acid sequence represented by the consensus sequence, all the amino acid sequences represented by the consensus sequence are modified differently. In some examples, one amino acid residue may contain multiple modifications, such as one, two or three modifications. In some examples, all the modifications on the same amino acid residue are of the same type. In some other examples, at least some of the modifications on the same amino acid residue are of different types. Examples of such modification include, but are not limited to glycosylation, sulfation, phosphorylation, ubiquitination, methylation, lipidation, biotinylation, hydroxylation and acetylation.

[0049] The term "glycosylation" as used herein refers to the addition of a glycosyl group, usually to, but not limited to an arginine, an asparagine, a cysteine, a hydroxylysine, a serine, a threonine, a tyrosine, or a tryptophan residue, resulting in a glycoprotein. A glycosyl group refers to a substituent structure obtained by removing the hemiacetal hydroxyl group from the cyclic form of a monosaccharide and, by extension, of a lower oligosaccharide.

[0050] The term "sulfation" as used herein refers to the addition of a sulfo group usually to a tyrosine residue. A sulfo group refers to group SO_3H^-, derived from sulfuric acid.

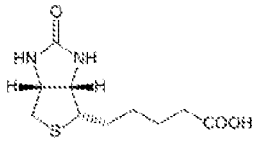
[0051] The term "phosphorylation" as used herein refers to the addition of a phosphate group. Protein phosphorylation commonly takes place at the serine, threonine, tyrosine,

arginine, lysine, or cysteine residues. Phosphorylation may alter the structural conformation of a protein, causing it to become activated, deactivated, or modifying its function.

[0052] The term "ubiquitination" as used herein refers to the addition of a ubiquitin. Ubiquitin is a small protein that is found in almost all cellular tissues in humans and other eukaryotic organisms, which helps to regulate the processes of other proteins in the body. The genes in the human genome that produce ubiquitin include, but are not limited to: *UBB*, *UBC*, *UBA52* and *RPS27A*. Ubiquitination may affect, for example, the activity and location of a protein, as well the interaction of the ubiquitinated protein with other proteins. In some examples, ubiquitination may lead to the degradation of the protein. In some examples, the protein is degraded via the proteasome.

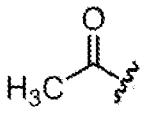
[0053] The term "methylation" as used herein refers to the addition of a methyl group. A methyl group refers to an alkyl derived from methane, containing one carbon atom bonded to three hydrogen atoms, that is $-CH_3$. Protein methylation can commonly take place at the arginine or lysine amino acid residues.

[0054] The term "lipidation" as used herein refers to the addition of a lipid group. Examples of lipidation include but are not limited to N-Myristoylation, Palmitoylation, Glycosylphosphatidylinositol (GPI)-anchor addition and Prenylation.

[0055] The term "biotinylation" as used herein refers to the addition of a biotin. A biotin refers to 5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoic acid, with the following structural formula: 

.

[0056] The term "hydroxylation" as used herein refers to the addition of a hydroxyl group (-OH).

[0057] The term "acetylation" as used herein refers to the addition of an acetyl group. An acetyl group contains a methyl group single-bonded to a carbonyl, represented by the following formula: 

.

[0058] Amino acid changes in the polypeptide or fragment thereof may be effected by techniques well known to persons skilled in the relevant art. For example, amino acid changes may be effected by nucleotide replacement techniques which include the addition, deletion or substitution of nucleotides, under the proviso that the proper reading frame is

maintained. Exemplary techniques include random mutagenesis, site-directed mutagenesis, oligonucleotide-mediated or polynucleotide-mediated mutagenesis, deletion of selected region(s) through the use of existing or engineered restriction enzyme sites, and the polymerase chain reaction.

[0059] The cell internalization of the cytotoxic peptide disclosed herein may require facilitators such as cell penetrating or cell targeting peptides or proteins. Thus, in one example, the isolated cytotoxic peptide as described herein is conjugated to at least one internalizing peptide or protein.

[0060] The term "conjugate" as used herein refers to the attachment of the isolated cytotoxic peptide to another object, such as other peptide(s) or polypeptide(s), protein(s), antibody (ies), or particle(s). The attachments can be resulted from covalent bonding or weak interactions. The term "conjugation" should be construed accordingly.

[0061] In some examples, the isolated cytotoxic peptide as described herein can be conjugated to an antibody.

[0062] The term "antibody" is used in the broadest sense to refer to an immunoglobulin molecule, a fragment of an immunoglobulin molecule, or a derivative of either thereof. The antibody can be a monoclonal antibody or a polyclonal antibody. In some examples, the antibody is an antibody for cancer therapy. Examples of an antibody for cancer therapy include, but are not limited to, 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). Other antibodies for cancer therapy can also be used.

[0063] The isolated cytotoxic peptide as described herein can be conjugated to a protein or an antibody by, for example but not limited to, a cross-linker, a bifunctional linker or a trifunctional linker. Linker groups can be, for example but not limited to, small organic compounds or peptides substituted with chemical linkers. In some examples, the chemical linkers can be, but not limited to, any one of the following: succinimidyl 3-(2-

pyridyldithio)propionate (SPDP), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB),4-succinimidyloxycarbonyl-alpha-methyl-α(2-pyridyldithio)toluene (SMPT), EMCS (N-ε-malemidocaproyl-oxysuccinimide ester) and Sulfo-MBS (m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester).

[0064] In some examples, a thiol group on the peptide is required for conjugating the isolated cytotoxic peptide as described herein to a linker. Thus, the isolated cytotoxic peptide will have at least one additional cysteine residue at either end of the peptide. Therefore, in some examples, the isolated cytotoxic peptide as described herein further comprises at least one cysteine residue at the N-terminal and/or the C-terminal end.

[0065] In general, the isolated cytotoxic peptide as described herein can exert its cytotoxic effect on any cell type, once the isolated cytotoxic peptide is internalized into the cell. Therefore, depending on the type of internalizing peptide or protein being conjugated to, the isolated cytotoxic peptide as described herein can exert its cytotoxic effect on a specific type of cell. In some examples, the internalizing peptide or protein is a cell penetrating peptide or protein. In some other examples, the internalizing peptide or protein is a cell targeting peptide or protein. In some examples, a cell penetrating peptide or protein can also function as a cell targeting peptide or protein. Examples of peptides that can function as both a cell penetrating peptide and a cell targeting peptide include but are not limited to, LTVSPWY peptide (LTV, SEQ ID NO: 8), HLYVSPW peptide (Pep2, SEQ ID NO: 795), CGFYWLRSC peptide (NRP, SEQ ID NO: 796) and CQDGRMGFC peptide (BLA, SEQ ID NO: 797). In some specific examples, the cell targeting peptide or protein is a cancer cell targeting peptide or protein. In some other specific examples, the cell targeting peptide or protein is a lipocyte targeting peptide or protein. In some further examples, the cell targeting peptide or protein is a stem cell targeting peptide or protein. In some examples, such conjugation can result in the internationalization of both the cytotoxic peptide and the internalizing peptide or protein. Examples of a cell-penetrating peptide include, but are not limited to, 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), FGF (AAVALLPAVLLALLAP) (SEQ ID NO: 798) and R6W3 (RRWWRRWRR)(SEQ ID NO: 7). Examples of a cancer cell-targeting peptide include but

are not limited toarginine-glycine-aspartic acid (RGD), asparagine-glycine-arginine (NGR), TCP-1 phage peptide (TCP-1), and the peptides listed in Table 1. Examples of a lipocyte-targeting peptide include but are not limited to, CKGGRAKDC peptide (ATS, SEQ ID NO: 39). Examples of stem cell targeting peptide are listed in Table 2. Examples of conjugated peptides and their effects are discussed in details in Examples 6 to 8.

SEQ ID NO:	Cancer cell targeting peptide
8	LTVSPWY
9	IFLLWQR
10	CGNSNPKSC
11	SVSVGMKPSPRP
64	CSNIDARAC
65	CDPSRGKNC
66	CPSDLKDAC
67	CRTTRGTKC
68	CRMTRNKPC
69	CRVSRQNKC
70	CAKIDPELC
71	CGGERGKSC
72	YSINDWH
73	YSFNSWM
74	PNPNNST
75	YPTPYDI
76	LPAMPNS
77	CNRRTKAGC
78	SRHDLNS
79	STVATSQ
80	QRLGNQWAVGHLM
81	RGDF
82	KGVSLSYR
83	QFPPKLTNNSML
84	SYDILKPNPQRL
85	SHGKPPSFSPYT
86	LLADTTHHRPWT
87	CTPSPPFSHC
88	CPNGRC
89	CLSYYPSYC
90	RTRYED
91	GMMYRS
92	RWRTNF
93	RLQLKL
94	RIPLEM
95	QFDEPR
96	TSAVRT

97	GLWQGP
98	QCTGRF
99	LPGMMG
100	DVGTTE
101	TDLGAM
102	DSNAES
103	ITDMAA
104	WRPCES
105	WRNTIA
106	IDKQLE
107	FMEIET
108	HEVVAG
109	GGHTRQ
110	INGKVT
111	VPWXEPAYQRFL
112	GRDS
113	RGEPAYQRFL
114	RGDPAYQRFL
115	WXEPAYQGRFL
116	WXEPAYNGRFL
117	RGEPAYQGRFL
118	RGDPAYQGRFL
119	RGEPAYNGRFL
120	RGDPAYNGRFL
121	WXEPAYQRFL
122	AXEPAYQRFL
123	WAEPAYQRFL
124	WXAPAYQRFL
125	WXEAAYQRFL
126	WXEPAAQRFL
127	WXEPAYARFL
128	WXEPAYQAFL
129	WXEPAYQAAL
130	WXEPAYQAFA
131	EXEPAYQRFL
132	LXEPAYQRFL
133	KXEPAYQRFL
134	QXEPAYQRFL
135	YXEPAYQRFL
136	FXEPAYQRFL
137	F*XEPAYQRFL
138	WXEPAYQREL
139	WXEPAYQRLL
140	WXEPAYQRKL
141	WXEPAYQRQ
142	WXEPAYQRYL

143	WXEPAYQRF*L
144	WXEPAYQRFL
145	WXEPAYQRRE
146	WXEPAYQRFK
147	WXEPAYQRFQ
148	WXEPAYQRFT
149	WXEPAYQRFF
150	XEPAYQRFL
151	XEPAYQREL
152	XEPAYQRLL
153	XEPAYQRKL
154	XEPAYQRQL
155	XEPAYQRYL
156	XEPAYQRF*L
157	XEPAYQRFL
158	XEPAYQRFE
159	XEPAYQRFK
160	XEPAYQRFQ
161	XEPAYQRFT
162	XEPAYQRFF
163	KSLSRHDHIHHH
164	GGCLQILPTLSECFGR
165	GLKVCGRYPGICDGIR
166	GKYTWYGYSLRANWMR
167	VPCQKRPGWVCLW
168	KWCVIWSKEGCLF
169	SSWCMRGQYNKICMW
170	VECYLIRDNLCIY
171	WWCLGERVVRCAH
172	FYCVIERLGVCLY
173	RVCFLWQDGRCVF
174	NRLKCRAQATHSAAPCIRGY
175	RQNSCTYSDARRWALCWSGE
176	QLNSCIHSGDRAIRGCMDWV
177	KYGLCRDETVFPSHSCTFTG
178	GSPQCPGGFNCPRCDCGAGY
179	GTGSCGYGKLHTGYWCSYFP
180	NSSSCDTSVVRSTWACILQP
181	VRAVCTTLKSRGHEECWSLQ
182	VYAQCGVNVRTGRGGCSRLM
183	VHMNCSWMRVSEGHPCESAD
184	GRQGCYEHLWRLIAWCAIFL
185	LRMTCAFGVAQRSADCALSS
186	SIVNCSAALTDLPTRCGGNI
187	CGTRCVRCQNGPEASCEQPL
188	TPLFCGNHGRQPSPLCMKWD

189	FTTVCRQPRGHEAIVCGSGK
190	APSFCGTAMLGASRYCYSGP
191	GARECESGGPGMRKLCTQIN
192	NNRACFRTSKGNPAECPYLG
193	GSLACQNIVVCVKKQCNALC
194	KRASCQNPLFSNFFVCGLSE
195	LPNFCMDTSGRAGPLCMGSE
196	RHTVCRVSLSSVQGSCSHEY
197	CGLIIQKNEC
198	CNAGESSKNC
199	GRRTRSRRLRRS
200	SMSIASPQIPWS
201	TPRNLRTSNTHR
202	GRRIAGPYIALE
203	SMPINSPYIPWS
204	GRRPMKLNKTP
205	GRRINRLILPRN
206	GRRTRSSRLRNS
207	CLSDGKRKC
208	CLDGGRPKC
209	CREAGRKAC
210	CAGRRSAYC
211	CNRRTKAGC
212	CPIEDRPMC
213	CGRRAGGSC
214	CGNSNPKSC
215	CPHNLTKLC
216	GPLPLR
217	CDCRGDCFC
218	GHGKHKNK
219	HKHGHGHGKHKNKGK
220	KHGHGHGK
221	KGHHGKHG
222	HKNKGKKN
223	CRGRRST
224	CRSRKG
225	CKAAKNK
226	CKGAKAR
227	FRVGVADV
228	CEYQLDVE
229	CSRPRRSEC
230	CGKRK
231	CDTRL
232	CGTKRKC
233	CDTAVVEGL
234	CRSRKG

235	CEYQLDVE
236	CPIEDRPMC
237	PIEDRPM
238	PIDERPM
239	ALRDRPM
240	PMMRQRPM
241	PLASRPM
242	PEKFRPM
243	VPEQRPM
244	DLPMHPM
245	QFQSQPM
246	QPPMEYS
247	NGRSL
248	MTQMIS
249	TALSPQ
250	WNLPWYYSVSPT
251	LTVLPW
252	LTVEPWL
253	LTVSPLWD
254	LTVTPWL
255	LTVQPWP
256	LTVSPWT
257	VLTVQPW
258	LTVSLWT
259	PGVIPWN
260	LTYQTWP
261	ELYVSRL
262	NLYYASW
263	TLTVLPW
264	NLYVASW
265	SMSIARL
266	VSFLEYR
267	CPGPEGAGC
268	RGDfK
269	PRPGAPLAGSWPGTS
270	ADGAPRPGAPLA
271	DRWRPALPVVLFPLH
272	ASSSYPLIHWRPWAR
273	DRWRPALP
274	IHWRPWAR
275	AAEWLDAFFVRHVDR
276	GDVWLFLTSTSHFAR
277	GCSVSSVGALCTHV
278	APCCSHLDASPFQRP
279	AQSNFVTWGYNVAV
280	RASDVGSDVVPRYPF

281	MARSGL
282	MARAKE
283	MSRTMS
284	MTKSAG
285	MTKCRG
286	MTRNLQ
287	MTRQIG
288	MSRPHK
289	MAKHAM
290	CWWRLEGC
291	CLQLFSTC
292	CAKGYRSC
293	CTGSWLGC
294	AEGEFMYWGDSHWLQYWYEGDPAK
295	AEGEFWGDSHWLQYWYEGDPAK
296	AEGEFIHNRYNRFFYWYGDPAK
297	AEGEFPRWGDSHWLQYWYEGDPAK
298	AEGEFLMWGGSHWLEYWYEGDPAK
299	AEGEFGHWCDQHWLQYWYEGDPAK
300	AEGEFGWWGDSHWLQYEGDPAK
301	CRGDCF
302	CDCRGDCFC
303	CNGRCVSGCAGRC
304	CGSLVRC
305	CRGDCGGKWCFRVCYRGICYRRCR
306	MCPKHPLGC
307	LCPKHPLGC
308	HLQIQPWYPQIS
309	VPWMEPAYQRFL
310	LSSVNSFPVVTP
311	QPWLEQAYYSTF
312	SALLPWPVLVNY
313	ITTPWDEMRSFL
314	HSFLHPWDLFDY
315	VPWMEPAYQRFL
316	MLPKPSSFPVPG
317	HSFLHPWDLFDY
318	CNGRCVSGCAGRC
319	CVLNGRMEC
320	FDDARL
321	FSDARL
322	FSDMRL
323	FVDVRL
324	FTDIRL
325	FNDYRL
326	FSDTRL

327	DPAFIFYHSTLFFNS
328	GGHDGDPVLTGTLFY
329	AVDPRMFYLLLRGGA
330	PIHYIF
331	YIHYIF
332	RIHYIF
333	WREWFL
334	WWAMKP
335	LILSSGELLRHPRG
336	TAASGVRSMH
337	LTLRWVGLMS
338	GGGTRAGMKY
339	WGKIEDPLRA
340	AGQTLTASGD
341	DLLAVSWLRA
342	SAERGVVAMS
343	AIHSELMWVS
344	FWTERAGWAY
345	MVWSKGPLFL
346	AGTRMSWEVL
347	VSRSSRWGSI
348	DAHVLVPRTP
349	AQGIVLQLAL
350	LSPLLSPATA
351	CDCRGDCFC
352	CNGRCVSGCAGRC
353	NGRAHA
354	CVLNGRMEC
355	HGRFILPWWYAFSPS
356	RFRGLISLSQVYLSP
357	ARVSFWRYSSFAPTY
358	GSWYAWSPLVPSAQI
359	KKEKDIMKKTI
360	GRGDSPK
361	SNPFSKPYGLTV
362	YPHYSLPGSSTL
363	KDEPQRRSARLSAKPAPPKPEPKPKKAPA KK
364	TLTYTWS
365	CREKA
366	CGQKRTRGC
367	RPARPAR
368	CRGDGWC
369	RGDGWK
370	TSPLNIHNGQKL
371	CRGDKGPDC

372	CRGDRGPDC
373	CRGDKTTNC
374	CRGDHAGDC
375	CRGDHGVEC
376	CGRGDNLPC
377	CGRGDNLAC
378	CEKRGDNLC
379	CEKRGDSVC
380	CSGRGDSLC
381	CGKRGDSIC
382	CTGRGDALC
383	CRGDSAC
384	CRGDKGENC
385	CGRGDSPDC
386	CRGDKGPEC
387	CRGDKHADC
388	CRGDHAANC
389	CRGDAGINC
390	CGRGDMPSC
391	CEKRGDSLC
392	KDEPQRRSARLSAKPAPPKPEPKPKKAPA KK
393	CREKA
394	TDSILRSYDWTY
395	DMPKQLLAPWYY
396	DMPKQLLAPWYY
397	SYPLSFLGPLIS
398	TQQPLEGHQLPY
399	TGVSWSVAQPSF
400	SVSVGMKPSPRP
401	SQWNSPPSSAAF
402	CGNSNPKSC
403	SFSIIHTPILPL
404	GNGRAHA
405	AHLPIVRASLPS
406	TPMNHHSQHAER
407	GNIIPDRPMHPT
408	FPSSLIIPPLPN
409	EDIKPKTSLAFR
410	YEDIKPKTSLAFR
411	TQPADLQTHNHN
412	FDHSSKWTRTSP
413	YSHNTITNLYFS
414	WPRYAESTLQLR
415	KGVSLSYRKKGVSLSYR
416	SVSVGMKPSPRP

417	WPLHTSVYPPSP
418	NTLPPFSPPSPP
419	SFPDSNIAPSSP
420	QHAPSNSKSVLT
421	WPTYLNPSSLKA
422	GPSGNLHIRPAS
423	SPLLSTRAVQLS
424	SPMFTMIQGDAQ
425	VNSHQALWSPAQ
426	STLPPPLRFANV
427	SFNQPYLYKTAF
428	YHTRIALPDNLP
429	AQSTAFQKPLLM
430	KCCYSL
431	RLLDTNRPLLPY
432	CSDSWHYWC
433	FQHPSFI
434	SMSIASPYIALE
435	SMSIASPYIPWS
436	SPGPMKLLKTPL
437	TLNINRLILPRT
438	SMSIGSPYITFG
439	VPNTNSLPAAVN
440	LIAKTALPQTNK
441	LIAKTALPQTN
442	CPHSKPCLC
443	GGCRGDMFGC
444	FRPNRAQDYNTN
445	QEFSPYMGLEFKKH
446	QEFSPNLWGLEFQKN
447	QEYSPNLWGHEFRSH
448	HTFEPGV
449	PSTNHAL
450	PSTLTSS
451	APSQTYH
452	KAMSWYA
453	SRESPHP
454	QSRLSLG
455	LDHFAPM
456	LDKKTTS
457	NMSPQLD
458	SQRQTLD
459	STKLLHE
460	TSPTNRS
461	PHSPTSL
462	HGKYFVS

463	PQRHVNY
464	MMSQLAH
465	PMAHLEF
466	ELIKESR
467	QPENLPT
468	NTHMTAF
469	PFKLSKH
470	ASSLHTI
471	HPLRLPA
472	HQSVNKE
473	LQNPTPE
474	PTEAQLQ
475	LFAQLGP
476	NQPTRAL
477	TPRTQKA
478	IHFPSAS
479	PLRIAQH
480	CRLTGGKGVGC
481	CRRTNWQGAGC
482	CQLTGTHGAGC
483	CADPNSVRAMC
484	CADPNSVRAHC
485	CAAHYRVGPWC
486	PQNSKIPGPTFLDPH
487	SMEPALPDWWWKMFK
488	DKPTAFVSVYLKTAL
489	APRPGPWLWSNADSV
490	GVTDSSTSNLDMPHW
491	PKMTLQRSNIRPSMP
492	LYPLHTYTPLSLPLF
493	LTGTCLQYQSRCGNTR
494	AYTKCSRQWRTCMTTH
495	ANTPCGPYTHDCPVKR
496	NISRCTHPFMACGKQS
497	PRNICSRRDPTCWTTY
498	GCNGRC
499	QHWSYKCIRP
500	CVSNPRWKC
501	CHVLWSTRC
502	SWLAYPGAVSYR
503	YSAYPDSVPMMS
504	SRESPHP
505	VPWMEPAYQRFL
506	CDSDSDITWDQLWDLMK
507	ATLDGVS
508	RRHSVSG

509	SGWFAGS
510	GSVSHRR
511	GSVLPVL
512	RSGRVSN
513	NSVRGSR
514	NVVRQ
515	CDCRGDCFC
516	CSDSWHYWC
517	CSDWQHPWC
518	CSDYNHHWC
519	CSDGQHYWC
520	CYDSWHYWC
521	CFDGNHIWC
522	CTDFPRSFC
523	CTQDRQHPC
524	CLSRYLDQC
525	CPRECESIC
526	CTTHWGFTLC
527	CRRHWGFEFC
528	CVPELGHEC
529	HTMYYHHYQHHL
530	CGNKRTRGC
531	CGRECPRLCQSSC
532	CGEACGGQCALPC
533	IWSGYGVYW
534	PSCAYMCIT
535	WESLYFPRE
536	SKVLYYNWE
537	CGLMCQGACFDVC
538	CERACRNLCREGC
539	CPRGCLAVCVSQC
540	CKVCNGRCCG
541	CEMCNGRCMG
542	CPLCNGRCAL
543	CPTCNGRCVR
544	CGVCNGRCGL
545	CEQCNGRCGQ
546	CRNCNGRCEG
547	CVLCNGRCWS
548	CVTCNGRCRV
549	CTECNGRCQ
550	CRTCNGRCLE
551	CETCNGRCVG
552	CAVCNGRCGF
553	CSCCNGRCGD
554	CWGCNGRCRM

555	CPLCNGRCAR
556	CKSCNGRCLA
557	CVPCNGRCHE
558	CQSCNGRCVR
559	CRTCNGRCQV
560	CVQCNGRCAL
561	CRCCNGRCSP
562	CASNNGRVVL
563	CGRCNGRCLL
564	CWLCNGRCGR
565	CSKCNGRCGH
566	CVWCNGRCGL
567	CIRCNGRCSV
568	CGECNGRCVE
569	CEGVNGRRLR
570	CLSCNGRCPS
571	CEVCNGRCAL
572	GRSQMQI
573	HHTRFVS
574	SKGLRHR
575	VASVSVA
576	WRVLAAF
577	KMGPKVW
578	IFSGSRE
579	SPGSWTW
580	NPRWFWD
581	GRWYKWA
582	IKARASP
583	SGWCYRC
584	ALVGLMR
585	LWAEMTG
586	CWSGVDC
587	DTLRLRI
588	SKSSGVS
589	IVADYQR
590	VWRTGHL
591	VVDRFPD
592	LSMFTRP
593	GLPVKWS
594	IMYPGWL
595	CVMVRDGDC
596	CVRIRPC
597	CQLAAVC
598	CGVGSSC
599	CVSGPRC
600	CGLSDSC

601	CGEGHPC
602	CYTADPC
603	CELSLISKC
604	CPEHRSLVC
605	CLVVHEAAC
606	CYVELHC
607	CWRKFYC
608	CFWPNRC
609	CYSYFLAC
610	CPRGSRC
611	CRLGIAC
612	CDDSWKC
613	CAQLLQVSC
614	CYPADPC
615	CKALSQAC
616	CTDYVRC
617	CGETMRC
618	CLSGSLSC
619	WGTGLC
620	GICKDDWCQ
621	TSCDPSLCE
622	KGCGTRQCW
623	YRCREVLCQ
624	CWGTGLC
625	WSCADRTCM
626	AGCRLKSCA
627	SRCKTGLCQ
628	PICEVSRCW
629	WTCRASWCS
630	GRCLLMQCR
631	TECDMSRCM
632	ARCRVDPCV
633	CIEGVLGGC
634	CSVANSC
635	CSSTMRC
636	SIDSTTF
637	GPSRVGG
638	WWSGLEA
639	LGTDVRQ
640	LVGVRLL
641	GRPGDJW
642	TVWNPVG
643	GLLLVVP
644	FAATSAE
645	WCCRQFN
646	VGFGKAL

647	DSSLRLP
648	KLWCAMS
649	SLVSFLG
650	GSFAFLV
651	IASVRWA
652	TWGHLRA
653	QYREGLV
654	QSADRSV
655	YMFWTSR
656	LVRRWYL
657	TARGSSR
658	TTREKNL
659	PKWLLFS
660	LRTNVVH
661	AVMGLAA
662	VRNSLRN
663	TDCTPSRCT
664	SWCQFEKCL
665	VPCRFKQCW
666	CTAMRNTDC
667	CRESLKNC
668	CMEMGVKC
669	VTCRSLMCQ
670	CNNVGSYC
671	CGTRVDHC
672	CISLDRSC
673	CAMVSMED
674	CYLGVSNC
675	CYLVNVDC
676	CIRSAVSC
677	LVCLPPSCE
678	RHCFSQWCS
679	FYCPGVGCR
680	ISCAVDAC
681	EACEMAGCL
682	PRCESQLCP
683	RSCIKHQCP
684	QWCSRRWCT
685	MFCRMRSCD
686	GICKDLWCQ
687	NACESAICG
688	APCGLLACI
689	NRCRGVSCT
690	FPCEGKKCL
691	ADCRQKPCL
692	FGCVMASCR

693	AGCINGLCG
694	RSCAEPWCY
695	DTCRALRCN
696	KGCGTRQCW
697	GRCVDGGCT
698	YRCIARECE
699	KRCSSSLCA
700	ICLLAHCA
701	QACPMLLCM
702	LDCLSELCS
703	AGCRVESC
704	HTCLVALCA
705	IYCPGQECE
706	RLCSLYGCV
707	RKCEVPGCQ
708	EDCTSRFCS
709	LECVVDSCR
710	EICVDGLCV
711	RWCREKSCW
712	FRCLERVCT
713	RPCGDQACE
714	CNKTDGDEGVTC
715	PQRRSARLSA
716	IELLQAR
717	ISLLQAR
718	IDLMQAR
719	IILLQGR
720	ISLLGAR
721	FSLLDAR
722	CTPSPFSHC
723	AGTRMSWEV
724	CSNRDARRC
725	WQPDTAHHWATL
726	HNAYWHWPPSMT
727	GHLIPLRQPSH
728	YTSPHHSTTGHL
729	WTHHHSYPRPL
730	NSFPLMLMHHHP
731	KHMHWHPPALN
732	SLDSMSPQWHAD
733	SEFIHHWTPPPS
734	NGFSHHAPLMRY
735	HHEWTHHWPPP
736	AWPENPSRRPF
737	AGFQHHPSFYRF
738	QRSPMMSRIRLP

739	YRHWPIDYPPP
740	MIHTNHWWAQD
741	CALIIQKNEC
742	CGLILQKNEC
743	CGLIIQRNEC
744	CGLIINKNEC
745	CNAAESSKNC
746	CNAGESSRNC
747	CNAGESTKNC
748	CNAGDSSKNC
749	CLSDGK
750	CLSDGKPVS
751	CSMSAKKKC
752	CKTRVSCGV
753	CASLSCR
754	CSGGKVLDC
755	CASLSCR
756	CSGGKVLDC
757	CSMSAKKKC
758	CKTRVSCGV
759	CASLSCR
760	WIFPWIQL
761	WDLAWMFRLPVG
762	CRGSGAGRC
763	CKGGRAKDC
764	CGSPGWVRC
765	VGVGEWSV
766	SRPRR
767	CXSRPRRZC
768	CSRPRRSVC
769	CSRPRRSWC
770	CGLSGLGVA
771	CPIRPMEDC
772	CPIDERPMC
773	CALRDRPMC
774	CPEKFRPMC
775	CSPQSQPMC
776	CGLIIQKNEC
777	CRGDK
778	KDEPQRRSARLSAKPAPPKPEPKPKKAPA KK
779	CGQKRTRGC
780	NVVRQ
781	PHSCNK
782	HPLSKHPYWSQP
783	IFLLWQR

784	APRPG
785	CRGDRCPDC
786	CVNHPAFAC
787	KDEPQRRSARLSAKPAPPKPEPKPKKAPA KK
788	CGKRK
789	KDEPQRRSARLSAKPAPPKPEPKPKKAPA KKC

Table1 . Sequences of cancer cell targeting peptides

SEQ ID NO:	Stem cell targeting peptide sequence
41	AVGPYSGLKVFR
42	SPLSKGSTHLST
43	TNKPVQPRQTLP
44	AMHPSPASAKMN
45	AGAPYRNTNAGT
46	TPTERFNTHHVE
47	QAGDEKEWLGPK
48	GSNQSVRYLQQT
49	NVDYAFGKREQS
50	DPLLHSQADVQS
51	LPHSSWNPKLAL
52	HQVHAKPLDLMP
53	QYTFSVNPLMRA
54	VPHFSTPTSVFQ
55	NYAIAVVNVLSH
56	NTKVPDPTARLL
57	HGAAWGTRTGHV
58	VPATETAQAGHA
59	VEYHFNHTMTAY
60	VGGEAWSSPTDL
61	HSDQVALKMTRS
62	QPHSVSVSDTWH
63	SNNDNLAHRVRL

Table 2. Sequences of stem cell targeting peptides

[0066] The isolated cytotoxic peptide or conjugate thereof as described herein can exert its cytotoxicity selectively. Thus, in some examples, the isolated cytotoxic peptide or conjugate thereof has low toxicity towards cells and tissue that are not the target of the conjugated cell penetrating peptide or cell targeting peptide.

[0067] In some examples, the isolated cytotoxic peptide or conjugate thereof exerts its cytotoxic effects only upon internalization into a target cell.

[0068] The stability of the isolated cytotoxic peptide as disclosed herein can be enhanced by conjugating to a particle, in particular a small particle. Examples of a particle include but are not limited to, nanoparticle or microparticle. In some examples, the nanoparticle or microparticle is made from materials including but not limited to, metal, silica, carbon, polymeric materials, and mixtures thereof. Examples of metal include, but are not limited to, gold and silver. Examples of polymeric materials include, but are not limited to, bio-degradable polymers such as a poly(lactide-co-glycolide), poly(lactic acid), poly(alkylene glycol), polybutylcyanoacrylate, poly(methylmethacrylate-co-methacrylic acid), poly-allylamine, polyanhydride and polyhydroxybutyric acid.

[0069] In the present disclosure, there is provided an isolated nucleic acid molecule comprising the nucleotide sequence encoding a peptide as described herein.

[0070] Since the degeneracy of the genetic code permits substitutions of certain codons by other codons which specify the same amino acid and hence give rise to the same protein, the disclosure is not limited to a specific nucleic acid molecule but includes all nucleic acid molecules comprising a nucleotide sequence coding for the peptide as described herein.

[0071] A nucleic acid molecule, such as DNA (including cDNA and genomic DNA), RNA (such as mRNA), is regarded to be 'capable of expressing a nucleic acid molecule or a coding nucleotide sequence' or capable 'to allow expression of a nucleotide sequence' if it contains regulatory nucleotide sequences which contain transcriptional and translational information and such sequences are "operably linked" to nucleotide sequences which encode the peptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequences sought to be expressed are connected in such a way as to permit gene sequence expression. The precise nature of the regulatory regions needed for gene sequence expression may vary from organism to organism, but shall, in general include a promoter region which, in prokaryotes, contains only the promoter or both the promoter which directs

the initiation of RNA transcription as well as the DNA sequences which, when transcribed into RNA will signal the initiation of synthesis. Such regions will normally include non-coding regions which are located 5' and 3 ' to the nucleotide sequence to be expressed and which are involved with initiation of transcription and translation such as the TATA box, capping sequence and CAAT sequences. These regions can for example, also contain enhancer sequences or translated signal and leader sequences for targeting the produced peptide to a specific compartment of a host cell, which is used for producing a peptide described above. The isolated nucleic acid molecule as described herein can be obtained by cloning or produced synthetically. Examples of the isolated nucleic acid molecules are listed in Table 3.

SEQ ID NO:	Sequences (5' to 3')
790	cagctgggcaaaaaaaaacatcgccgccgcccgagcaaaaaaaaacgccattgg
791	cagctgggcgcggcggcgcatcgccgccgcccgagcaaaaaaaaacgccattgg
792	cagctgggccgccgccgccatcgccgccgcccgagccgccgccgccgccattgg
793	cagctgggcaaaaaaattctggcggcgcgcccgagcaaaaaaaaacgccattgg
794	catcgccgccgcccgagcaaaaaaaaacgccattgg

Table 3. Examples of isolated nucleic acid molecules encoding the polypeptides as described herein

[0072] The isolated nucleic acid molecule comprising the nucleotide sequence encoding the peptide as disclosed herein can be comprised in a vector, for example an expression vector. Thus, in the present disclosure, there is provided a vector comprising an isolated nucleic acid molecule as described herein. Representative vectors include plasmids, cosmids, and viral vectors. Vectors can also comprise nucleic acids including expression control elements, such as transcription/translation control signals, origins of replication, polyadenylation signals, internal ribosome entry sites, promoters and enhancers, wherein the control elements are operatively associated with a nucleic acid encoding a gene product. Selection of these and other common vector elements are conventional and many such sequences can be derived from commercially available vectors. A vector can be introduced into targeting cells using any suitable method known in the art for introducing DNA into

cells, including but not limited to microinjection, electroporation, calcium phosphate precipitation, liposome-mediated delivery, viral infection, protoplast fusion, and particle-mediated uptake.

[0073] The vector comprising the isolated nucleic acid molecule can be transformed into host cells capable of expressing the genes. The transformation can be carried out in accordance with standard techniques. Thus, the disclosure is also directed to a (recombinant) host cell containing a vector as described herein. In this context, the transformed host cells can be cultured under conditions suitable for expression of the nucleotide sequence encoding the peptide as described above. Host cells can be established, adapted and completely cultivated under serum free conditions, and optionally in media which are free of any protein/peptide of animal origin. Commercially available media such as RPMI-1640 (Sigma), Dulbecco's Modified Eagle's Medium (DMEM; Sigma), Minimal Essential Medium (MEM; Sigma), CHO-S-SFMII (Invitrogen), serum free-CHO Medium (Sigma), and protein-free CHO Medium (Sigma) are exemplary appropriate nutrient solutions. Any of the media may be supplemented as necessary with a variety of compounds, examples of which are hormones and/or other growth factors (such as insulin, transferrin, epidermal growth factor, insulin like growth factor), salts (such as sodium chloride, calcium, magnesium, phosphate), buffers (such as HEPES), nucleosides (such as adenosine, thymidine), glutamine, glucose or other equivalent energy sources, antibiotics, trace elements. Any other necessary supplements may also be included at appropriate concentrations that are known to those skilled in the art.

[0074] The isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule or the vector as described herein can be formulated into compositions, for example pharmaceutical compositions, suitable for administration. Where applicable, the peptide or conjugate thereof, the nucleic acid molecule or the vector can be administered with a pharmaceutically acceptable carrier. A "carrier" can include any pharmaceutically acceptable carrier as long as the carrier is compatible with other ingredients of the formulation and not injurious to the patient. Accordingly, pharmaceutical compositions for use may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Thus, in one example, the present disclosure provides a pharmaceutical composition comprising, but not limited to, an isolated cytotoxic peptide or

conjugate thereof, an isolated nucleic acid molecule or a vector as described herein. In another example, the pharmaceutical composition further comprises one or more pharmaceutically acceptable excipients, vehicles or carriers.

[0075] Examples of pharmaceutically acceptable excipients, carriers or diluents are demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oil, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysolpoxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or iso-propanol; lower aralkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerin; fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate; polyvinylpyrolidone; agar; gum tragacanth or gum acacia, and petroleum jelly. Typically, the carrier or carriers will form from 10% to 99.9% by weight of the compositions.

[0076] The isolated cytotoxic peptide or conjugate thereof as described herein may be present in the compositions in any of a wide variety of forms. For example, two, three, four or more peptides or conjugates thereof may be merely mixed together or may be more closely associated through complexation, crystallization, or ionic or covalent bonding. The peptides or conjugates thereof can also be formulated as prodrugs.

[0077] The term “prodrug” as used herein refers to compounds that rapidly convert *in vivo* into pharmacologically active compounds. Suitable prodrugs can be made, for instance, by conjugating the isolated cytotoxic peptide as described herein to one or more protective peptides. Various linkers known in the art can be used in such conjugations, for example but not limited to valine-citrulline dipeptide linker. Such linkers can be cleavable, resulting in the release of the pharmacologically active compounds. For example, valine-citrulline dipeptide linker is cleavable by lysosomal cathepsin B, resulting in the release of the cytotoxic peptide.

[0078] The isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule and the vector as described herein can also be used in combination with one or more other therapeutic agents to achieve better results of treatment and/or to reduce potential

side effects. Thus, in one example, the pharmaceutical composition further comprises one or more therapeutic agent. The isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule and/or the vector as described herein may be administered simultaneously, sequentially or separately from the one or more further therapeutic agent. By simultaneous is meant that the isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule or the vector as described herein may be contained in the same pharmaceutical composition as the one or more further therapeutic agent, or they are contained in different pharmaceutical compositions but taken at the same time. By sequential is meant that the isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule or the vector as described herein and the one or more further therapeutic agent are contained in different pharmaceutical compositions, and are administered one immediately after another. By separately is meant the isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule or the vector as described herein and the one or more further therapeutic agent are contained in different pharmaceutical compositions, and are administered with a period of time apart. For example, they can be administered separately with about 12 hours, 10 hours, 8 hours, 6 hours, 4 hours, 2 hours, 1 hour, 30 minutes, or 15 minutes apart. In some examples, when the isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule or the vector as described herein and the one or more further therapeutic agent are contained in different pharmaceutical compositions, they can be administered using different routes.

[0079] The one or more therapeutic agent can be therapeutic agent against cancer, therapeutic agent for weight loss, or therapeutic agent with other effects, including but not limited to, reducing inflammation, reducing infection and relieving pain. In one example, the one or more therapeutic agent is a therapeutic agent against cancer, including but not limited to, general chemotherapeutic agents such as alkylating agents, antimetabolites, anti-tumor antibiotics, topoisomerase inhibitors, mitotic inhibitors and corticosteroids, inhibitors of translation and transctiption; immunotherapeutic agents such as antibodies, in particular monoclonal antibodies, cytokines and vaccines; and targeted therapeutic agents such as small molecule drugs. In another example, the one or more therapeutic agent is a therapeutic agent for weight loss, including but not limited to, benzphetamine, bupropion hydrochloride, diethylpropion, lorcaserin, methamphetamine, naltrexone hydrochloride, orlistat, phendimetrazine, phentermine and sibutramine.

[0080] The isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule, the vector or the pharmaceutical composition as described herein can be administered in a number of ways depending upon whether local or systemic administration is desired and upon the area to be treated. For example, administration may be oral, intraadiposal, intraarterial, intraarticular, intracranial, intradermal, intralesional, intramuscular, intranasal, intraocularal, intrapericardial, intraperitoneal, intrapleural, intraprostatical, intrarectal, intrathecal, intratracheal, intratumoral, intraumbilical, intravenous, intravesicularl, intravitreal, liposomal, local, mucosal, enteral, parenteral, rectal, subconjunctival, subcutaneous, sublingual, topical, transbuccal, transdermal, vaginal, in crèmes, in lipid compositions, via a catheter, via a lavage, via continuous infusion, via infusion, via inhalation, via injection, via local delivery, via localized perfusion or any combination thereof. One skilled in the art will be able to identify the method to adapt the pharmaceutically active component to the characteristics suitable for the selected route of administration. Such characteristics include, but are not limited to, solubility, stability, and deliverability.

[0081] Compositions and formulations for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

[0082] Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions that may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

[0083] Compositions as described herein include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

[0084] The formulations as described herein, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general the formulations are prepared by uniformly and intimately bringing into association the active

ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

[0085] The compositions as described herein may be formulated into any of many possible dosage forms including, but not limited to tablets, capsules, liquid syrups and soft gels. The compositions as described herein may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances that increase the viscosity of the suspension including, for example, sodium carboxymethyl cellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0086] The compositions as described herein may additionally contain other adjunct components conventionally found in pharmaceutical compositions. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present disclosure, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present disclosure. The formulations can be sterilized and, if desired, mixed with auxiliary agents, including but not limited to, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the antibody(s) of the formulation.

[0087] The isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule, the vector or the pharmaceutical composition as described herein can be used in the treatment or prevention of diseases. Examples of such diseases include, but are not limited to, cancer and obesity. Thus, in one example, there is provided a method of treating or preventing, for example, but not limited to cancer and obesity, in a subject comprising administering an isolated cytotoxic peptide as described herein, an isolated nucleic acid molecule as described herein, a vector as described herein or a pharmaceutical composition as described herein.

[0088] In another example, there is provided an isolated cytotoxic peptide or conjugate thereof as described herein, an isolated nucleic acid molecule as described herein, a vector as

described herein or a pharmaceutical composition as described herein, for use in treating or preventing, for example, but not limited to cancer and obesity, in a subject.

[0089] In yet another example, there is provided use of an isolated cytotoxic peptide or conjugate thereof as described herein, an isolated nucleic acid molecule as described herein or a vector as described herein in the manufacture of a medicament for treating or preventing, for example, but not limited to cancer and obesity, in a subject.

[0090] The major types of cancers that can be treated include but are not limited to carcinoma, sarcoma, lymphoma, germ cell tumor and blastoma. The specific types of cancers that can be treated include but are not limited to 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 cancer (for example, lip, tongue, mouth, and pharynx), and rhabdomyosarcoma. In one specific example, the cancer is breast cancer.

[0091] In some examples, the cancer is invasive and/or metastatic cancer. In some other examples, the cancer is stage I cancer, stage II cancer, stage III cancer or stage IV cancer.

[0092] Stem cell therapy is a promising treatment for various diseases such as cancers, cardiovascular diseases, brain and spinal cord injury, blood related diseases, Parkinson's disease, Alzheimer's disease, diabetes, wound healing and so forth. The major types of stem cells include but are not limited to, embryonic stem cells, induced pluripotent stem cells and tissue stem cells. One main concern of the stem cell therapy, in particular the therapy using embryonic stem cells or induced pluripotent stem cells, is that the transplantation of any undifferentiated stem cells can lead to side effects such as the formation of teratoma, which is a tumor containing tissues derived from all three embryonic germ layers, i.e., ectoderm, mesoderm, and endoderm. To prevent the formation of teratoma resulted from stem cell

transplantation, drugs with specific cytotoxic effect against undifferentiated stem cells can be used.

[0093] Thus, in one example, the isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule, the vector or the pharmaceutical composition as described herein can be used in the prevention or treatment of diseases associated with stem cell transplantation. In some examples, the stem cell transplantation can be, but not limited to, transplantation of embryonic stem cells, transplantation of induced pluripotent stem cells, or transplantation of tissue stem cells, or a mixture thereof. One example of such diseases is teratoma. In some examples, the teratoma is benign, while in some other examples, the teratoma is malignant.

[0094] In another example, there is provided an isolated cytotoxic peptide or conjugate thereof as described herein, an isolated nucleic acid molecule as described herein, a vector as described herein or a pharmaceutical composition as described herein, for use in preventing or treating diseases associated with stem cell transplantation in a subject.

[0095] In yet another example, there is provided use of an isolated cytotoxic peptide or conjugate thereof as described herein, an isolated nucleic acid molecule as described herein or a vector as described herein in the manufacture of a medicament for preventing or treating diseases associated with stem cell transplantation in a subject.

[0096] In some examples, the subject that is being treated may be a mammal. In one specific example, the subject may be a human.

[0097] As used herein the terms “treatment”, “treating”, or other grammatical variants thereof, refers to any and all uses which remedy a disease state or symptoms, prevent the establishment of disease, or otherwise prevent, hinder, retard, or reverse the progression of disease or other undesirable symptoms in any way whatsoever. Treatments of cancer include but are not limited to: (i) the prevention or inhibition of cancer or cancer recurrence, (ii) the reduction or elimination of symptoms or cancer cells, and (iii) the substantial or complete elimination of the cancer in question. Treatment may be effected prophylactically (prior to the onset of the disease) or therapeutically (following diagnosis of the disease).

[0098] The isolated cytotoxic peptide or conjugate thereof, the isolated nucleic acid molecule, the vector or the composition as described herein may be provided in an amount that is therapeutically effective. The suitable amount includes a sufficient but non-toxic amount of the compound as described herein to provide the desired therapeutic effect. The

exact amount required will vary from subject to subject depending on factors such as the species being treated, the age and general condition of the subject, the severity of the condition being treated, the particular agent being administered, the mode of administration, and so forth, and may be determined by one of ordinary skill in the art using only routine experimentation.

[0099] Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. The administering physician can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of the composition. In general, dosage is from 0.01 μg to 100 g/kg of body weight, such as 0.01μg to 100g, 0.05μg to 90g, 0.1μg to 80g, 0.5μg to 70g, 1 μg to 60g, 10μg to 50g, 20μg to 40g, 30μg to 30g, 40μg to 20g, 50μg to 10g, 75μg to 5g, 100μg to 4g, 200μg to 3g, 300μg to 2g, 400μg to 1g, 500μg to 900mg, 600μg to 800mg, 700μg to 700mg, 800μg to 600mg, 900μg to 500mg, 1mg to 400mg, 2mg to 300mg, 3mg to 200mg, 3mg to 100mg, 4mg to 90mg, 5mg to 80mg, 6mg to 70mg, 7mg to 60mg, 8mg to 50mg, 9mg to 40mg, 10mg to 30mg or 15mg to 25mg/kg of body weight, and may be given once or more times daily, weekly, monthly or yearly. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the subject undergo maintenance therapy to prevent the recurrence of the disease state, wherein the peptide or composition is administered in maintenance doses, ranging from 0.01 μg to 100 g/kg of body weight, once or more times daily, weekly, monthly, yearly, to once every 2 years.

[00100] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the

present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[00101] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[00102] Other embodiments are within the following claims and non- limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

EXPERIMENTAL SECTION

[00103] Non-limiting examples of the invention will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

[00104] **Example 1 – HEXIM1 BR peptide induces cell death in a P-TEFb- and p53-independent fashion**

[00105] HEXIM1 was originally identified from vascular smooth muscle cells treated with HMBA, an anti-proliferation compound. HEXIM1 is known as the inhibitor of P-TEFb. P-TEFb is a protein complex, which is composed of cyclin-dependent kinase 9 (CDK9) and a cyclin partner (cyclin T1, T2a, T2b, or K) with cyclin T1 being the predominant CDK9-associated cyclin. P-TEFb controls the elongation phase of RNAPII transcription and is required for transcriptional regulation of human immunodeficiency virus.

[00106] HEXIM1 exerts its inhibitory function on P-TEFb only when associated with the 7SK snRNA, while neither 7SK snRNA nor HEXIM1 alone instigates any effects. It is hypothesized that association of the 7SK snRNA with HEXIM1 leads to a conformational change that renders the cyclin T1-binding domain of HEXIM1 accessible for P-TEFb binding. HEXIM1 contains several functional domains and the domains required for 7SK

snRNA and cyclin T1 binding have been identified. The N-terminus of HEXIM1, AA 1-150, has been characterized as a self-inhibitory domain (ID). Deletion of the ID enhances the inhibitory effects of HEXIM1 on P-TEFb activity. The region between AAs 150-180 of HEXIM1, which includes a stretch of basic residues, is referred to as the basic region (BR). The BR contains the binding motif for 7SK snRNA, KHRR (AAs 152-155). The P-TEFb binding motif, PYNT (AAs 202-205), is located between the BR and acidic region (AR, AAs 210-250). In the absence of 7SK snRNA, the AR can interact with the adjacent BR. Since the P-TEFb binding motif is located between the BR and AR, the BR-AR interaction may establish an auto-inhibitory conformation which prevents the association between HEXIM1 and P-TEFb. When 7SK snRNA binds to the BR, the BR-AR interaction is disrupted and the PYNT motif becomes accessible for P-TEFb binding. HEXIM1 can form a homodimer or a heterodimer with a HEXIM1-related protein, HEXIM2, through the dimerization domain (DD) at the C-terminus of HEXIM1.

[00107] When the 7SK snRNA binding motif, KHRR, is mutated to ILAA, the mutant HEXIM1 cannot interact with 7SK snRNA or regulate P-TEFb activity (Fig. 1B). It was found that FGF-BR-ILAA mutant still maintained its killing ability, in both HCT116 WT and p53 null cell lines, suggesting that P-TEFb might not be involved in the HEXIM1 BR-mediated cell killing (Fig. 1D).

[00108] HEXIM1 stabilizes p53 by blocking the HDM2-mediated ubiquitination of p53 and plays an essential role in p53 activation induced by anti-cancer drugs/compounds. HDM2, a p53-specific E3 ubiquitin ligase, is the master regulator of p53. Ubiquitination of p53 by HDM2 results in proteasome-mediated degradation of p53 protein. Six lysine residues, Lys-370, -372, -373, -381, -382, and -386, located within the C-terminal domain of p53 are known as the target residues for HDM2 ubiquitination. Six lysine residues located within the BR of HEXIM1 (Lys-150-152 and 159-161) are identified as the major sites for HDM2 ubiquitination. Sequence alignment of the ubiquitination sites between p53 (AAs 370-386) and HEXIM1 (AAs 150-161) exhibits similar distribution of the lysine residues (Fig. 1A). This suggests that HEXIM1 peptide containing these ubiquitination sites may have an impact on p53 activation, resulting in p53-dependnt cell arrest or cell death.

[00109] A commercial p53 activating peptide, FGF-p53, containing the p53 ubiquitination sites (AAs 361-382) fused with a cell membrane-translocating peptide derived from Kaposi fibroblast growth factor, was reported to induce apoptosis in mutant and wild-type p53-

bearing human cell lines. The internalized p53 peptide is likely to compete with the endogenous p53 protein in binding to HDM2 and protect the endogenous p53 from HDM2-mediated ubiquitination, resulting in stabilization and activation of p53. A FGF-BR fusion peptide was generated in the present disclosure, in which the cell penetrating FGF peptide was fused with HEXIM1 BR peptide containing the HDM2 ubiquitination residues (AAs 150-161). Two acute myeloid leukemia (AML) cell lines, AML2 and AML3, were treated with FGF-p53 and FGF-BR peptides and the effects of the peptides on cell viability were determined. FGF-p53 exhibited anti-proliferation effects on AML2 cells at high dosage (30 μM) but had no effects on AML3 cells (Fig. 11). The p53 level in AML2 has been shown to be significantly higher than that in AML3. This observation explained the p53-dependent anti-proliferation effects of FGF-p53 peptide in AML2 cells. However, FGF-BR peptide exhibited similar cytotoxicity on both cell lines and much stronger inhibition than FGF-p53 peptide, raising the possibility that the killing mediated by FGF-BR might not depend on p53 (Fig. 11).

[00110] Cytotoxicity was also observed in human cervical cancer cells, HeLa cells, when treated with FGF-BR peptide (Fig. 1C). Comparable cytotoxicity was also observed in normal cells, including HEK293 cells and human foreskin fibroblasts (HFFs) (Fig. 1C), suggesting the cytotoxic effect of BR peptide occurs to all cell types once it is internalized into cells.

[00111] The effect of FGF-BR peptide was also tested in human colon cancer cell line, HCT116 (p53 KO). The HCT116 (p53 WT) was used as a control. FGF-BR induced cell death in both HCT116 (p53 WT) and HCT116 (p53 KO) cell lines with similar potency, while the negative control peptide, FGF-X13, showed no effects in both cell lines (Fig. 1D). A fusion peptide, FGF-BR-RRR12 was generated, in which all six HDM2 ubiquitinating lysine residues were mutated to arginine and could no longer be ubiquitinated by HDM2 (Fig. 1B). If the BR peptide indeed induces cell death through the HDM2-p53 regulatory pathway, the BR-RRR12 mutant should lose its cytotoxic effects. FGF-BR-RRR12 did not exhibit reduced inhibitory effects but demonstrated stronger cell toxicity when compared to the wild-type FGF-BR peptide (Fig. 1D). It was also noticed that the p53 status had no effects on the cell killing mediated by FGF-BR-RRR12 (Fig. 1D), confirming that the cell death caused by the BR peptide was not p53-dependent.

[00112] **Example 2 – HEXIM1 BR induced cell death is independent of apoptosis**

[00113] The mechanism of cell death induced by the HEXIM1 BR peptide was examined. To monitor the real-time changes to the cells upon treatment with BR peptide, the effect of FGF-BR on HCT116 (p53 WT) and HCT116 (p53 KO) cells was examined in a live cell imaging setting. Cells treated with FGF peptide were included as a control. Within minutes, FGF-BR peptide rapidly induced drastic changes to the cell morphology with rupturing of the plasma membrane accompanied with damages to the nuclear membrane and abnormalities to the nucleolus in both cell types (Fig. 2A). No effects were detected when cells were treated with FGF peptide (Fig. 2A). Similar observations were seen in MCF7 breast cancer cells treated with LTV-tagged BR peptide, where BR peptide was conjugated to a breast cancer-targeting peptide, LTV (data not shown). Since BR-induced cytotoxicity occurred in minutes, whereas the duration of apoptosis is estimated to be from 12 to 24 hours, it is unlikely that FGF-BR lead to apoptosis. Moreover, the morphological changes seen in FGF-BR treated HCT116 cells do not resemble the characteristics of cells dying by apoptosis which include membrane blebbing and formation of apoptotic bodies. Cell swelling and subsequent rupturing of the plasma membrane followed by rapid lysis of the cells observed were descriptive of necrosis instead. In addition, the induction of cell death in LTV-BR-treated MCF7 cells could not be inhibited by a pan-caspase inhibitor z-VAD-Fmk (100 μM) (Fig. 2B), indicating that BR induced cell death is independent of apoptosis.

[00114] Taken together, it is unlikely that the conjugated FGF-BR peptide triggered apoptosis in the treated cells. Hence, using this BR peptide provides an attractive approach to eliminate cancer cells that have a defective apoptotic pathway.

[00115] **Example 3 – HEXIM1 BR induces rapid depolarization of mitochondrial membrane potential in a p53-dependent manner**

[00116] It has been reported that a cytotoxic basic peptide, KLA, elicit its killing action by disrupting the mitochondrial membrane potential (MMP), which is important for ATP generation and induction of apoptosis. Since HEXIM1 BR contains many basic residues, it is possible that the BR may utilize similar mechanism for its potent killing. MMP was measured using JC-1 dye after incubating with FGF-X13 or FGF-BR peptides for three minutes. Carbonylcyanide m-chlorophenylhydrazone (CCCP), a known mitochondrial membrane depolarizer, was used as a positive control. FGF-BR treated HCT116 (p53 WT) cells experienced rapid mitochondrial depolarization within three minutes (Fig. 3A), which is

similar to the time frame that morphological changes occurred upon addition of the FGF-BR peptide (Fig. 2A). However, no mitochondrial depolarization was observed in HCT116 (p53 KO) cells treated with FGF-BR (Fig. 3B), although these cells encountered rapid cytotoxicity similar to HCT116 (p53 WT) cells (Fig. 2A). It was known that p53 would translocate to the mitochondria, resulting in the reduction of MMP in p53-mediated apoptosis. Collectively, the data shows that HEXIM1 BR peptide is likely to induce cell killing though an alternative pathway that acts independently of p53 and apoptosis.

[00117] **Example 4 – HEXIM1 BR peptide alters subcellular localization of NPM and reduces its protein expression.**

[00118] NPM is a multi-functional protein and participates in the process of ribosome biogenesis. In addition to its role in protein translation, NPM is required to maintain DNA integrity in cells. Knockout of NPM results in accumulation of DNA damage, which clearly indicates the essential role of NPM in cell proliferation and survival. NPM is located in nucleolus, the sub-cellular site of ribosome synthesis and assembly. About 35% of AML patients carry the cytoplasmic-misallocated mutant form of NPM, NPMc+. NPMc+ is found to interact and sequester a portion of HEXIM1 in the cytoplasm of the NPMc+ AML cell line and activates P-TEFb-dependent transcription, suggesting the involvement of HEXIM1 in tumorigenesis of AML. The BR domain of HEXIM1 was known to contain a nucleolar localization sequence. When the BR was fused with yellow fluorescent protein (YFP), the BR-YFP was found to localize in nucleoli. NPM was identified as a HEXIM1 binding protein, and the BR of HEXIM1 was required for NPM binding. To determine the effects of FGF-BR peptide on NPM, immunofluorescence was performed to examine the sub-cellular distribution of NPM in the FGF-BR-treated HCT116 (p53 WT) and HCT116 (p53 KO) cells. Normal nucleolar localization of NPM was observed in control experiments (Fig. 4A, DMSO and FGF-X13), while mislocalization of NPM was detected in both cell types when incubated with FGF-BR (Fig. 4A, FGF-BR). Furthermore, in both cell types, a reduction in NPM protein level was observed in the FGF-BR treated cells as compared to the FGF-X13 control (Fig. 4B). Various post-translational modifications of p53, such as phosphorylation and acetylation, have been shown to stabilize and activate p53 in response to cellular stress. The expression levels of phosphorylation of p53 on Ser15 and acetylation of p53 on Lys382 were investigated, and it was found that they remained unchanged in HCT116 (p53 WT) cells when treated with FGF-BR peptide (data not shown), suggesting a p53-independent pathway

to trigger cell death. These results demonstrated that the BR peptide may interfere with protein translation/ribosome biosynthesis by disrupting sub-cellular localization of NPM and decreasing its expression, hence compromising its normal function.

[00119] **Example 5 – Selective killing of LTV-BR fusion peptide on breast cancer cells**

[00120] Therapeutic peptides can be divided into two major categories: cell targeting and cytotoxic peptides. Targeting peptides, including the cancer targeting peptides, provide selectivity and specificity to direct the targeted treatment. Cytotoxic peptides can be used as the toxin/payload in fusion peptides, nano-particle conjugates, and antibody drug conjugates (ADCs) for potential cancer treatment.

[00121] To explore the potential use of the cytotoxic HEXIM1 BR peptide in cancer therapy, a breast cancer targeting peptide, LTV, was fused to HEXIM1 BR and BR-RRR12 to generate the fusion peptide, LTV-BR and LTV-BR-RRR12 (SEQ ID NO: 16). Both LTV-BR and LTV-BR-RRR12 peptides exhibited anti-proliferation effects in breast cancer cell lines MCF7 and MDA-MB-231, while no effects were detected using the LVT-X13 control peptide (Fig. 5A and 5B). Although MDA-MB-231 cells are triple-negative with the absence of expression of oestrogen receptor, progesterone receptor and HER2, LTV-BR and LTV-BR-RRR12 elicited similar anti-cancer activity as compared to MCF7 cells (Fig. 5B). In addition, untagged control X13 (SEQ ID NO: 12) and the two BR peptides had no effect on cell viability (Fig. 5C and 5D).

[00122] To define the active region of the BR peptide, a series of truncated BR peptides were generated based on the stretches of basic residues found in the BR peptide sequence. These truncated peptides were fused to LTV (SEQ ID Nos: 22-26) and introduced to MDA-MB-231 cells. The results suggest that the region encompassing the second and third stretch of basic residues (HRRRPSKKKRHW) is more critical in exerting cytotoxic activity as compared to the first stretch of basic residues (Fig. 7). To confirm this hypothesis, stretches of basic residues were replaced by alanine residues in the active region of BR (see SEQ ID Nos: 27-33). Results shown in Fig. 8 demonstrated the importance of the same stretches of basic residues (HRRRPSKKKRHW) in BR induced cytotoxicity. Nonetheless, all three stretches of basic residues are required to exhibit the maximum cytotoxic effect against MDA-MB-231 cells.

[00123] KLA peptide (SEQ ID NO: 13), a cytotoxic peptide, was often fused with a cancer-targeting peptide or conjugated to antibodies recognizing cancer cells (as antibody-drug

conjugate, ADC) to exert cell-killing effect in anti-cancer therapy. LTV-KLA peptide (SEQ ID NO: 17) was generated and its effect was compared to that of LTV-BR. LTV-KLA exhibited stronger inhibition on the viability of MDA-MB-231 cells (Fig. 5B). However, it was noted that untagged KLA also showed non-specific killing on MDA-MB-231 cells, while BR- or BR-RRR12-treated cells remained highly viable (Fig. 5D). LTV-KLA also exhibited non-specific cell killing on non-breast cancer cell lines such as CHO (Chinese hamster ovary) and OPM-2 (multiple myeloma) cells, while little or no effects were observed in the LTV-BR-treated cells (Fig. 9A and 9B). Similar observations were seen for normal human fibroblasts HFF and WI-38, indicating the specificity of LTV-BR towards breast cancer cell lines but not LTV-KLA (Fig. 9C and 9D). It is predicted that a portion of the KLA ADCs will be broken down before reaching the target cancer cells, even though non-cleavable linkers may be used to generate these conjugates. As such, KLA peptides may be released from the conjugates, resulting in an off-target killing of normal cells/tissues. Taken together, the use of KLA as the toxic load in cancer therapy is questionable due to its non-specificity. In contrast, untagged HEXIM1 BR peptide is unable to kill cells when it is not fused with any cell penetrating or targeting peptides, suggesting that HEXIM1 BR peptide may be a safer alternative as compared to KLA, for the development of anti-cancer therapeutics.

[00124] **Example 6 – Cell penetrating/targeting peptide is required for internalization HEXIM1 BR peptide in cells**

[00125] The HEXIM1 BR peptide did not cause any cytotoxic effects when it was not fused with cell penetrating (for example, FGF) or cancer cell targeting (for example, LTV) peptides. It is thus possible that the unfused BR peptide may fail to internalize into cells without specific guidance. To test this hypothesis, fluorescent-labelled BR and LTV-BR peptides were generated and their presence in cells was examined by confocal microscope.

[00126] MCF7 cells were incubated with fluorescent-labeled peptides, followed by a washing step to remove the peptides failing to penetrate into cells. No fluorescent signals were observed in HEXIM1 BR peptide-treated cells (Fig. 6A), indicating that the BR peptide was not internalized. In the LTV-BR-incubated cells, fluorescent signals were detected within the cells (Fig. 6). LTV-BR was readily internalized into MCF7 cells and distributed in cytoplasm and nuclei (Fig. 6A). It was noted that its strong fluorescent signals were primarily localized in the nucleoli (Fig. 6A, LTV-BR-FITC). Detection of fluorescent signals in KLA-treated cells helps to explain the non-specific cytotoxicity induced by KLA peptide (Fig. 6A),

while no fluorescent signal was observed in HEXIM1 BR-treated cells, indicating that the BR peptide could not enter the cells by itself (Fig. 6A, BR-FITC). Cells treated with LTV-KLA demonstrated that the sub-cellular localization of the peptide was observed mainly in the cytoplasm (Fig. 6A). The different distribution of LTV-BR and LTV-KLA suggests that BR and KLA may utilize different mechanisms for cell killing.

[00127] Flow cytometric analysis was also performed to quantify the amount of internalized fluorescent peptide in MCF7 cells. LTV peptide directed the uptake of almost 100% of LTV-fused peptides (LTV-BR and LTV-KLA) into the breast cancer cell line (Fig. 6B). Approximately 65% of KLA-FITC-treated MCF7 cells internalized KLA-FITC, whereas there was no entry of BR-FITC into MCF7 cells (Fig. 6B). These results clearly demonstrate the safety feature of HEXIM1 BR peptide when compared to the non-specific cytotoxic KLA peptide. LTV assisted in the cellular internalization of HEXIM1 BR peptide into its target cells and the nucleolar localization of the fusion peptide might be subsequently guided by BR peptide.

[00128] **Example 7 – Application of HEXIM1 BR peptide in weight loss study**

[00129] $2.5x10^4$cells/ml of mouse fibroblast cells 3T3 were cultured in gelatin coated plates in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Gibco) to reach 100% confluent. The cells were then maintained at 100% confluency by supplementing with fresh media in a 37°C incubator with 5% CO_2 for 3 days. For induction, cells were then cultured in DMI Induction Media (which is DMEM supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, 0.1% 3-isobutyl-1-methylxanthine (sigma), 0.1% insulin (sigma) and 0.1% dexamethasone (sigma)) for 48 hours. The cells were then cultured in Insulin Induction Media (which is DMEM supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 0.1% insulin). The insulin induction media was changed every 2 days until the cells were differentiated to adipose cells.

[00130] A D-form BR peptide with the sequence of QLGKKKHRRRPSKKKRHW was tagged to a fat tissue targeting peptide of the sequence CKGGRAKDC (ATS peptide, SEQ ID NO: 39) to generate the ATS-BR_D peptide of the sequence CKGGRAKDCGG(QLGKKKHRRRPSKKKRHW)$_D$ (SEQ ID NO: 40). Both undifferentiated 3T3 cells and differentiated adipose cells were treated with the ATS-BR_D peptide. ATS-tagged KLA_D peptide was used as a positive control. Cell viability results

shown in Fig. 10 indicates that the ATS-BR_D peptide has no cytotoxic effect on undifferentiated 3T3 mouse fibroblast cells, but has significant cytotoxic effect on differentiated adipose cells. The cytotoxic effect of ATS-BR_D peptide on adipose cells is of similar potency as ATS-KLA_D peptide.

[00131] **Example 8 – Use of HEXIM1 BR peptide as the toxin for Antibody Drug Conjugates (ADCs)**

[00132] To generate ADCs, the cytotoxic HEXIM1 BR peptides are covalently attached to antibodies through chemical linkers. Cysteine and lysine are two most naturally occurring amino acids which are used to attach the toxin through the linker to the antibody. To conjugate at the lysine residues of antibodies, two chemical linkers, N-succinidyl-3-(2-pyridylothio)propionate (SPDP) and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), are utilized according to manufacturer's manual (Thermo Fisher Scientific). Briefly, the linkers are first crosslinked to the lysine residues of the antibodies. As the thiol group is required for conjugating the peptides to the chemical linkers, a cysteine residue is added at the N- or C-terminal of the BR peptide. After removing excess linkers and peptides, the effects of the BR-conjugated ADCs are analyzed *in vitro* using the selected breast cancer cell lines.

[00133] IgGs have four pairs of interchain disulfide bonds, two between the heavy chains in the hinge region and two on Fab between heavy and light chains. Intrachain disulfide bonds are to be left intact because they are critical to maintain the basic IgG domain structure essential for antigen recognition. Only partial reduction of the interchain disulfide bonds at the hinges gives eight potential conjugation sites through cysteine residues.

[00134] **Example 9 – Materials and Methods**

[00135] **Cell lines** – Human cell lines including HeLa, HEK293, MCF7, MDA-MB-231, CHO-K1, OPM-2, and WI-38 were obtained from American Type Culture Collection. AML2 and AML3 cells were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen. HCT116 p53+/+ and p53-/- cells were kindly given by Dr. Bert Vogelstein. Primary human foreskin fibroblasts (HFF) were obtained from Dr. Mark Stinski. HCT116, HCT116 (p53 KO), HeLa, 293, HFF, MCF7, CHO-K1, and WI-38 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Gibco). MDA-MB-231 and OPM-2 cells were

cultured in RPMI-1640 (Gibco) containing 10% FBS and 1% penicillin/streptomycin. All cells were routinely maintained in a 37°C incubator with 5% CO_2.

[00136] **Peptide Synthesis** – All peptides used in this study were chemically synthesized and purified by high performance liquid chromatography with >98% purity (First Base, Singapore). Their sequences are available in the Supplementary Table 1. Stock solutions were obtained by reconstituting the powder in sterile water or 50% DMSO and stored at -80°C.

[00137] **Immunoblotting analysis** – Cells were lysed in lysis buffer [50 mM Tris-HCl, (pH 7.5), 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, Protease Inhibitor tablet (Roche)] and used for western blotting. Western blotting was carried out as previously mentioned. The primary antibodies used include anti-NPM (Invitrogen) and anti-actin (Millipore). The film of western blot was scanned, and the protein bands were quantified by the GS-800 densitometer (Bio-Rad). The protein level of NPM was quantified after normalizing with the loading control, actin.

[00138] **Cell viability and cytotoxicity assays** – Cells were plated in clear-bottomed white walled 96-well plates (Corning) and incubated overnight. Cells were treated with indicated peptides in 1% FBS-containing media for overnight or indicated timings at 37°C. Cell viability was measured with CellTiter-Glo reagent (Promega) according to the manufacturer's instructions. For cytotoxicity assay, cells were plated in black walled 96-well plates (Corning) to allow them to adhere overnight. Upon treatment with a pan-caspase inhibitor, z-VAD-Fmk (100 μM) (Sigma) and subsequent treatment with LTV-tagged peptides, cytotoxicity induced was determined by CellTox cytotoxicity assay (Promega) 30 minutes after addition of peptides according to the manufacturer's instructions. Luminescence was determined using an Infinite 200 multiplate reader (Tecan).

[00139] **Measurement of MMP** – The cationic fluorescent dye 1, 1', 3, 3'-tetraethylbenzamidazolocarbocyanin iodide (JC-1) (Invitrogen) was utilized for MMP measurement. JC-1 was dissolved in DMSO (200 μM). Peptide-treated cells in 96-well deep sided, clear bottom, dark sided microplates were incubated with media containing JC-1 for 30 min at 37°C and then washed twice with warm PBS. Changes in MMP were determined using a multiplate spectrofluorometer (Tecan) (excitation: 475 nm; emission: 530nm (green); emission: 590nm (red)). The decrease in the ratio of red to green fluorescence was used to determine relative mitochondrial depolarization.

[00140] **Flow cytometry** – MCF7 cells were plated on 6-cm culture dish to allow overnight adherence. FITC-labeled peptides (30 μM) were added to the cells, incubated for 30 min at 37°C, and subsequently washed three times with PBS. The cells were then trypsinized, collected by centrifugation, and finally resuspended in 500μl ice-cold 2% FBS-containing PBS for flow cytometry analysis.

[00141] **Immunofluorescence and confocal microscopy** – For staining with anti-NPM, cultured cells were fixed in 10% neutral buffered formalin (Sigma) for 10 min, and then in methanol for 10 min, washed in PBS and incubated with blocking buffer (PBS containing 0.5% bovine serum albumin) for 1 hour prior to incubation with a mouse anti-NPM antibody (Invitrogen) in blocking buffer for overnight at 4°C. Cells were then incubated with Alexa Fluor 488-conjugated secondary antibody (Jackson Immuno Research Laboratories) for 1 hour, washed three times with PBS and counterstained with 4',6-diamidino-2-phenylindole (DAPI) -containing mounting solution (Vectashield). Stained cells were examined with a LSM 510 confocal microscope using a 63× objective lens (Zeiss).

[00142] To determine the ability of FITC-labeled peptides to enter the cells and to visualize intracellular distribution of the peptides, MCF7 cells were plated on 4-chamber glass cover slides (Lab-Tek) to adhere overnight, incubated with FITC-labeled peptides (30 μM) for 30min, and then washed three times with PBS before being fixed and mounted with DAPI-containing mounting solution (Vectashield). Images were acquired using a Nikon A1R confocal laser scanning microscope equipped with a 60× oil-immersion objective lens (SBIC-Nikon Imaging Centre).

[00143] **Statistical analysis** – All experiments were performed independently for at least three times. All statistical analyses for comparison between two groups were performed with two-tailed unpaired student's t-test using the Prism 5.01 (GraphPad Software).

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.

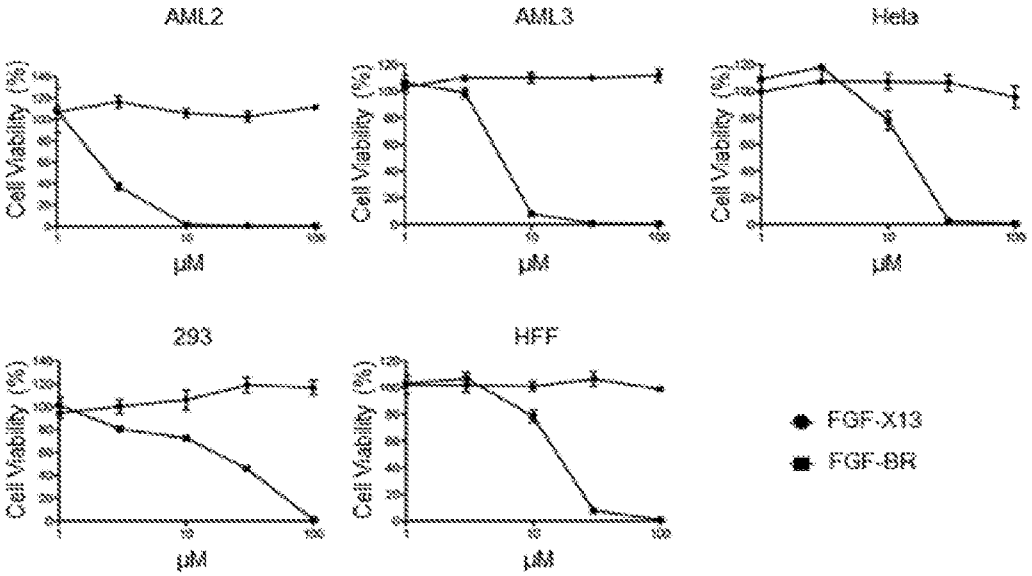
A

p53 (a.a. 370-386)	KSKKGQSTSRHKKLMFK	(SEQ ID NO: 34)
HEXIM1 (a.a. 150-161)	K-KKHRRRPS-KK---K	(SEQ ID NO: 35)

B

BR-WT	QLGKKKHRRRPSKKKRHW	(SEQ ID NO: 3)
BR-RRR12	QLGKKRHRRRPSRRRRHW	(SEQ ID NO: 4)
BR-iLAA	QLGKKILAARPSKKKRHW	(SEQ ID NO: 5)

C



D

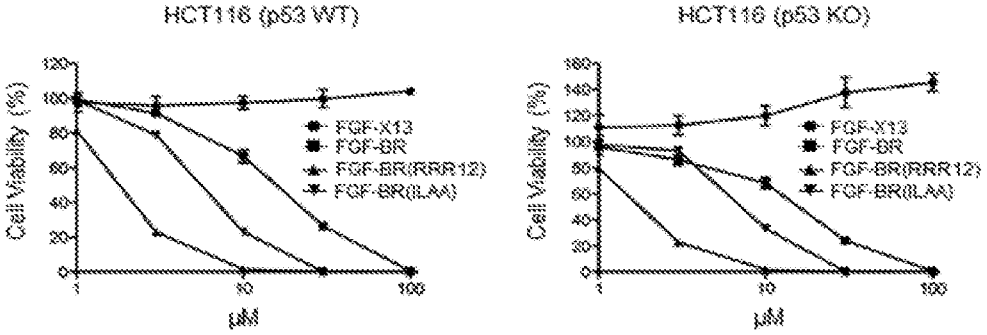


Fig. 1

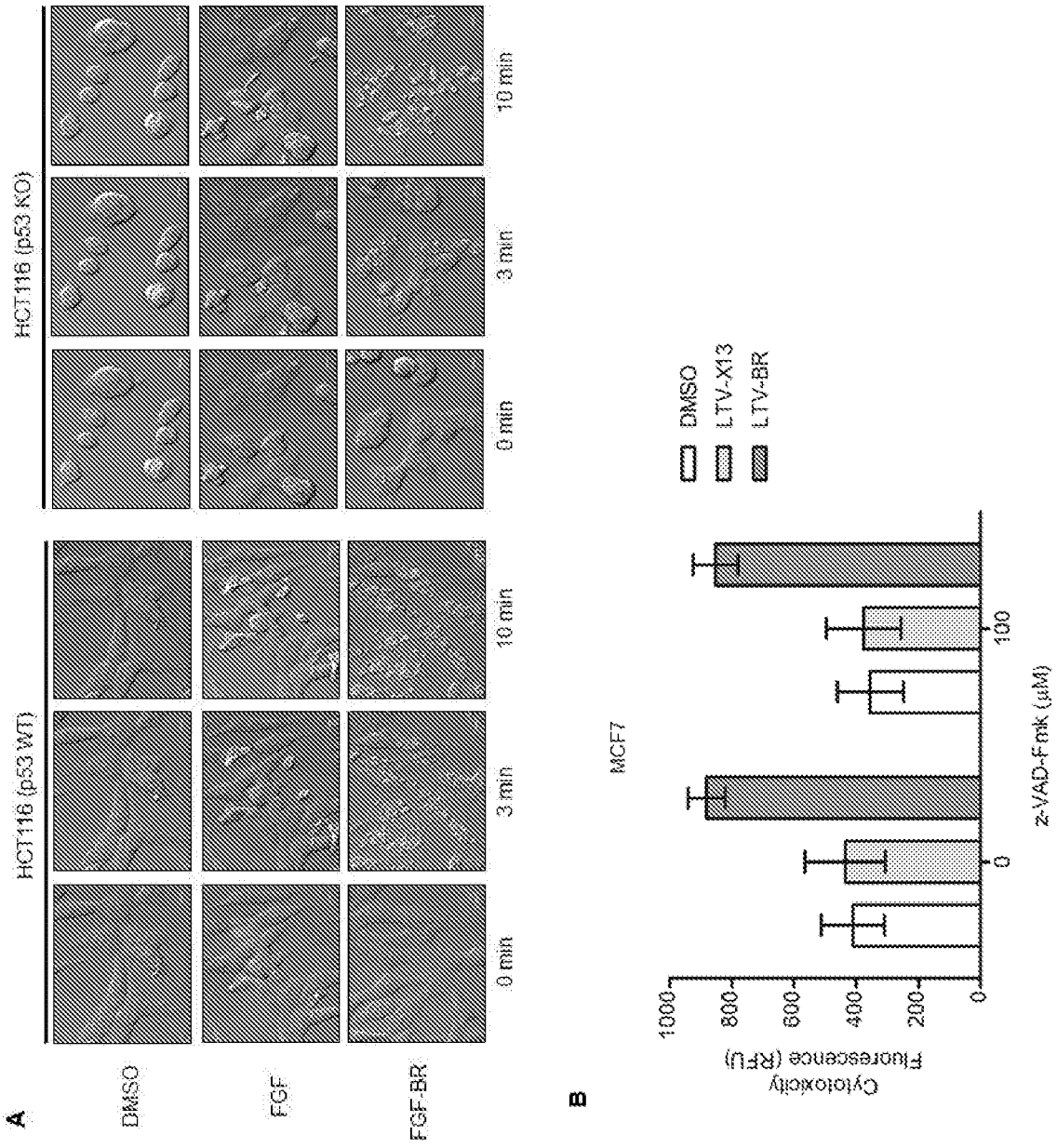

A
HCT116 (p53 WT)
HCT116 (p53 KO)
DMSO
FGF
FGF-BR
0 min
3 min
10 min
B
MCF7
DMSO
LTV-X13
LTV-BR
Cytotoxicity
Fluorescence (RFU)
1000
800
600
400
200
0
0
100
z-VAD-FMK (μM)

Fig. 2

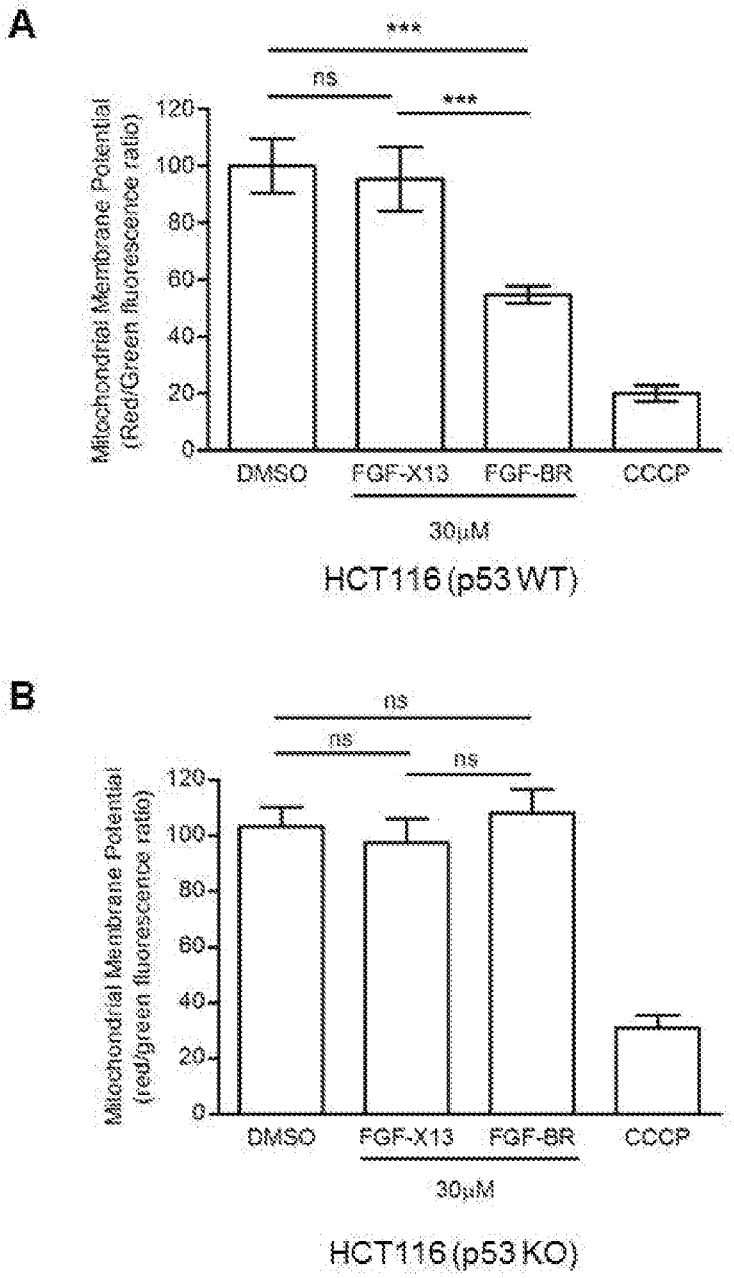

A
***
ns
***
Mitochondrial Membrane Potential (Red/Green fluorescence ratio)
120
100
80
60
40
20
0
DMSO
FGF-X13
FGF-BR
CCCP
30μM
HCT116 (p53 WT)
B
ns
ns
ns
Mitochondrial Membrane Potential (red/green fluorescence ratio)
120
100
80
60
40
20
0
DMSO
FGF-X13
FGF-BR
CCCP
30μM
HCT116 (p53 KO)

Fig. 3

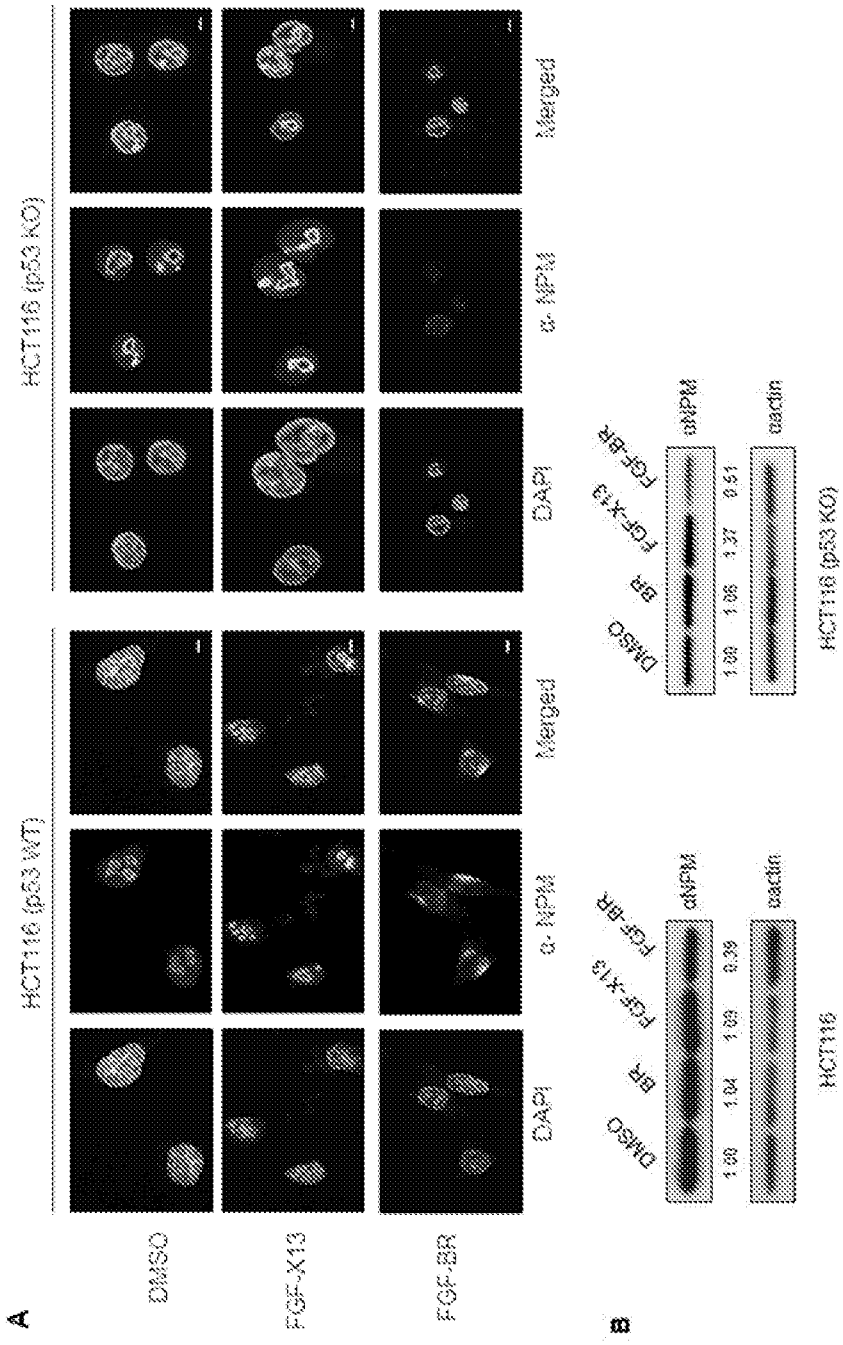

A
HCT116 (p53 WT)
HCT116 (p53 KO)
DMSO
FGF-X13
FGF-BR
DAPI
α- NPM
Merged
B
DMSO
BR
FGF-X13
FGF-BR
αNPM
actin
1.00
1.04
1.09
0.39
1.00
1.06
1.37
0.61
HCT116
HCT116 (p53 KO)

Fig. 4

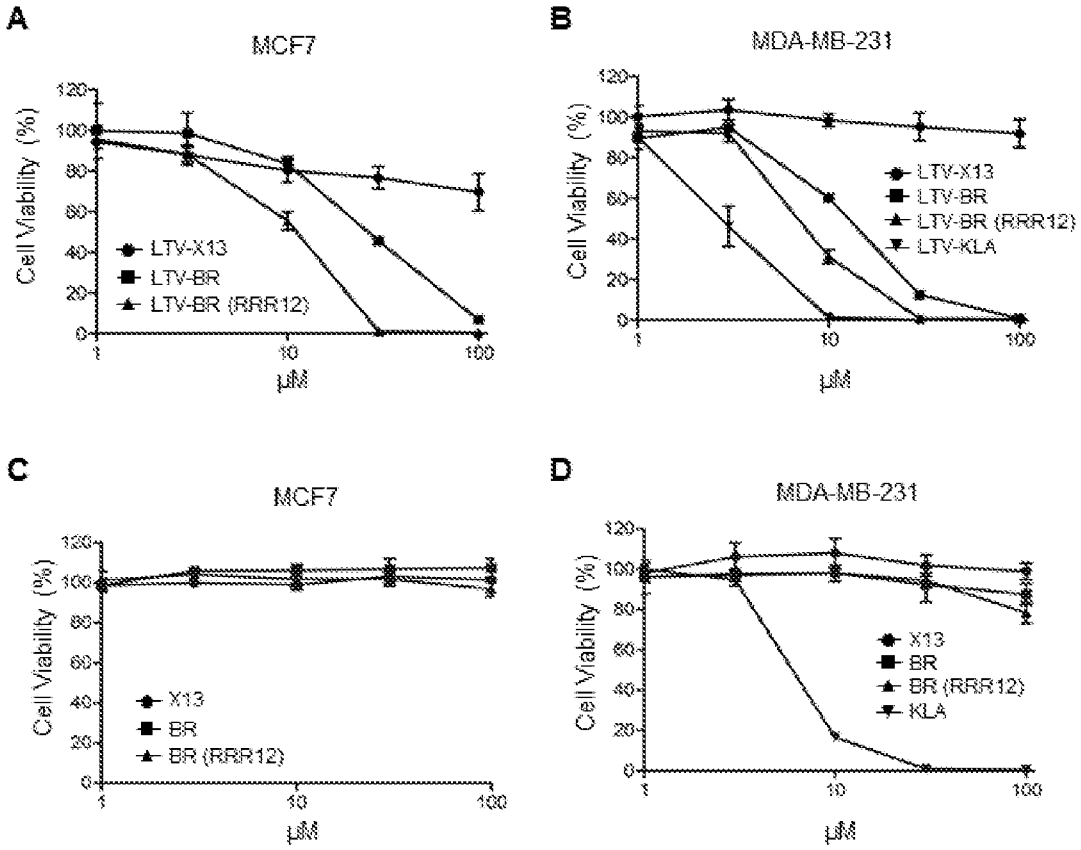

A
MCF7
Cell Viability (%)
LTV-X13
LTV-BR
LTV-BR (RRR12)
μM
B
MDA-MB-231
LTV-X13
LTV-BR
LTV-BR (RRR12)
LTV-KLA
C
MCF7
X13
BR
BR (RRR12)
D
MDA-MB-231
X13
BR
BR (RRR12)
KLA

Fig. 5

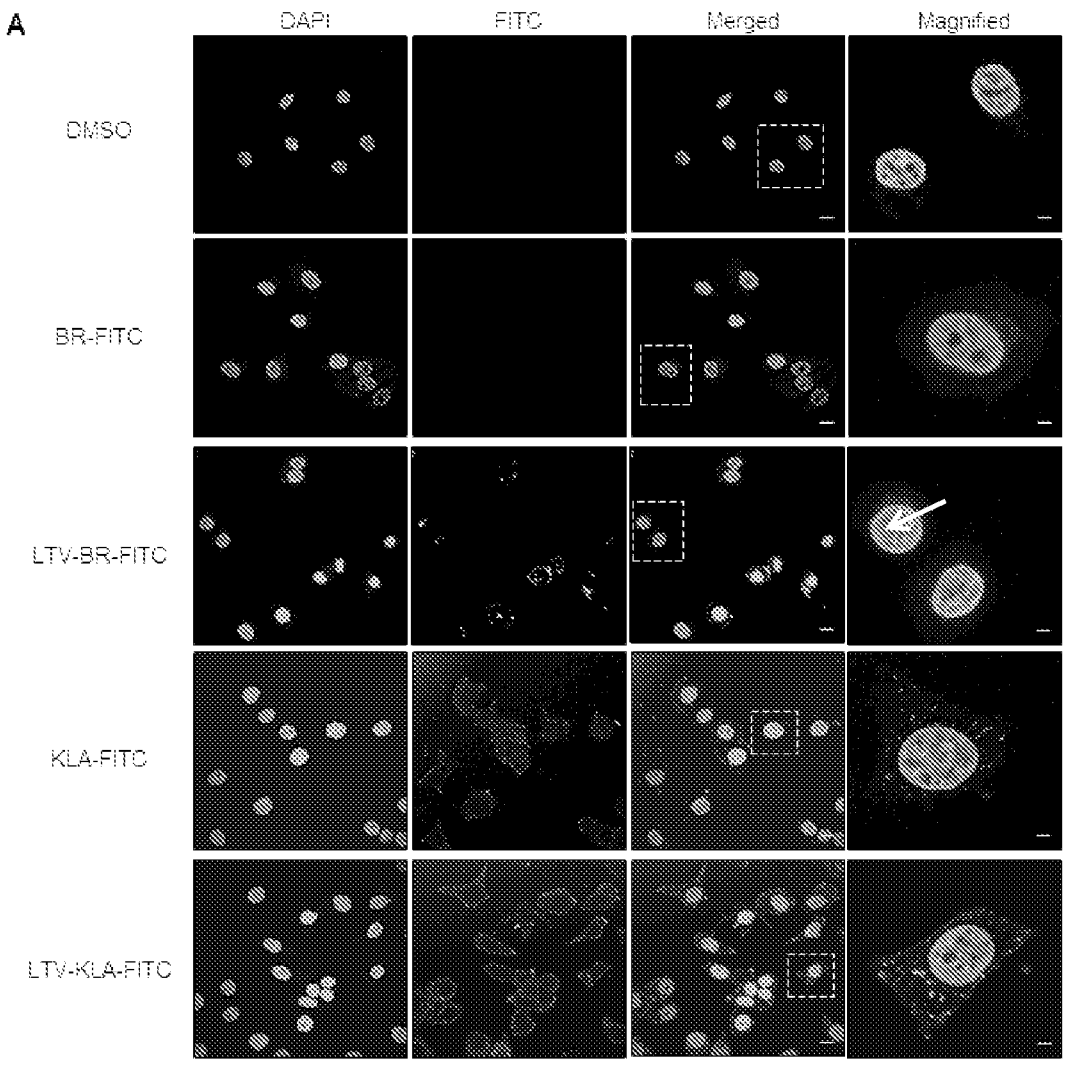

A
DAPI
FITC
Merged
Magnified
DMSO
BR-FITC
LTV-BR-FITC
KLA-FITC
LTV-KLA-FITC

Fig. 6

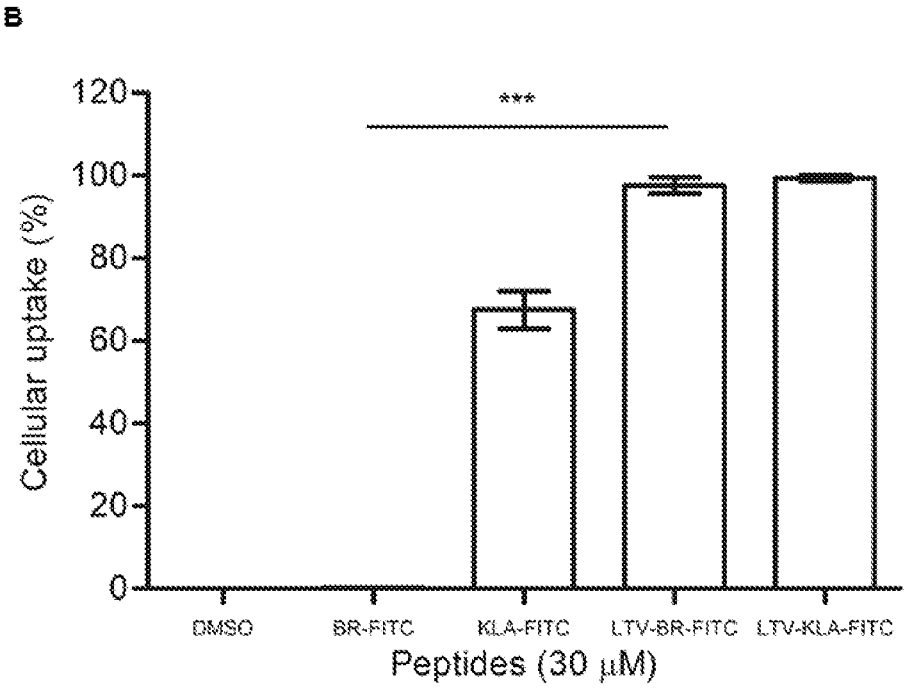
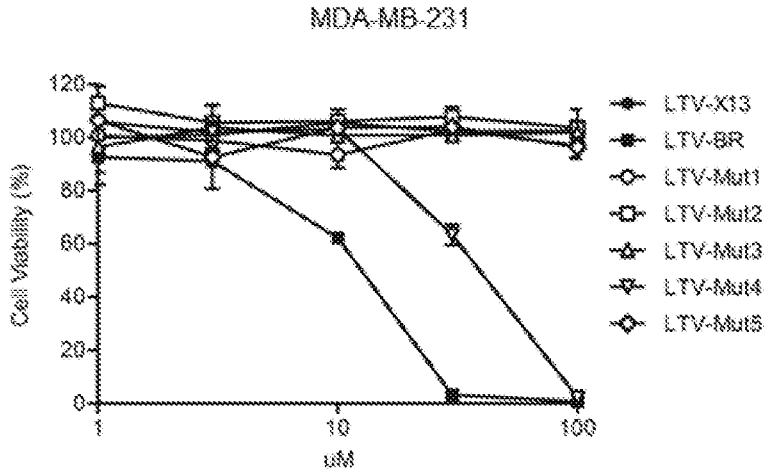
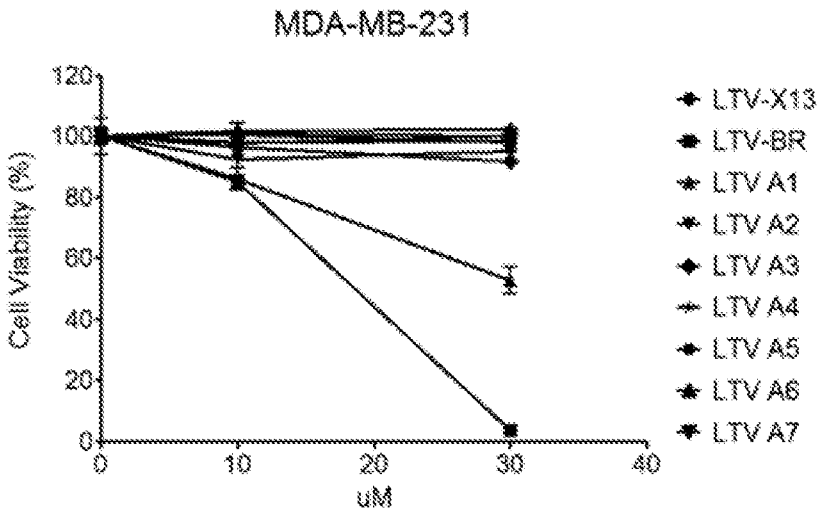


Fig. 6 (Continued)



Name	Sequence		Cell Killing Potency
LTV-BR	LTVSPWYGCGQLGKKRHRRRPSKKKRHW	(SEQ ID NO: 15)	++
LTV-Mut1	LTVSPWYGCGQLGKKRH----------	(SEQ ID NO: 22)	-
LTV-Mut2	LTVSPWYGCGQLGKKRHRRRPS-------	(SEQ ID NO: 23)	-
LTV-Mut3	LTVSPWYGCG----------PSKKKRHW	(SEQ ID NO: 24)	-
LTV-Mut4	LTVSPWYGCG--------HRRRPSKKKRHW	(SEQ ID NO: 25)	+
LTV-Mut5	LTVSPWYGCG--------HRRRPS-------	(SEQ ID NO: 26)	-

Fig. 7



Name	Sequence		Cell Killing Potency
LTV-BR	LTVSPWYGCGQLGKKRHRRPPSKKKRHN	(SEQ ID NO: 15)	++
LTV-BR(A1)	LTVSPWYGCGQLGAAAHRRRPSKKKRHN	(SEQ ID NO: 27)	+
LTV-BR(A2)	LTVSPWYGCGQLGKKRHAAAPSKKKRHN	(SEQ ID NO: 28)	-
LTV-BR(A3)	LTVSPWYGCGQLGKKRHRRRPSAAAAHN	(SEQ ID NO: 29)	-
LTV-BR(A4)	LTVSPWYGCGQLGAAARAAAPSKKKRHN	(SEQ ID NO: 30)	-
LTV-BR(A5)	LTVSPWYGCGQLGAAAHRRRPSAAAAHN	(SEQ ID NO: 31)	-
LTV-BR(A6)	LTVSPWYGCGQLGKKRHAAAPSAAAAHN	(SEQ ID NO: 32)	-
LTV-BR(A7)	LTVSPWYGCGQLGAAARAAAPSAAAAHN	(SEQ ID NO: 33)	-

Fig. 8

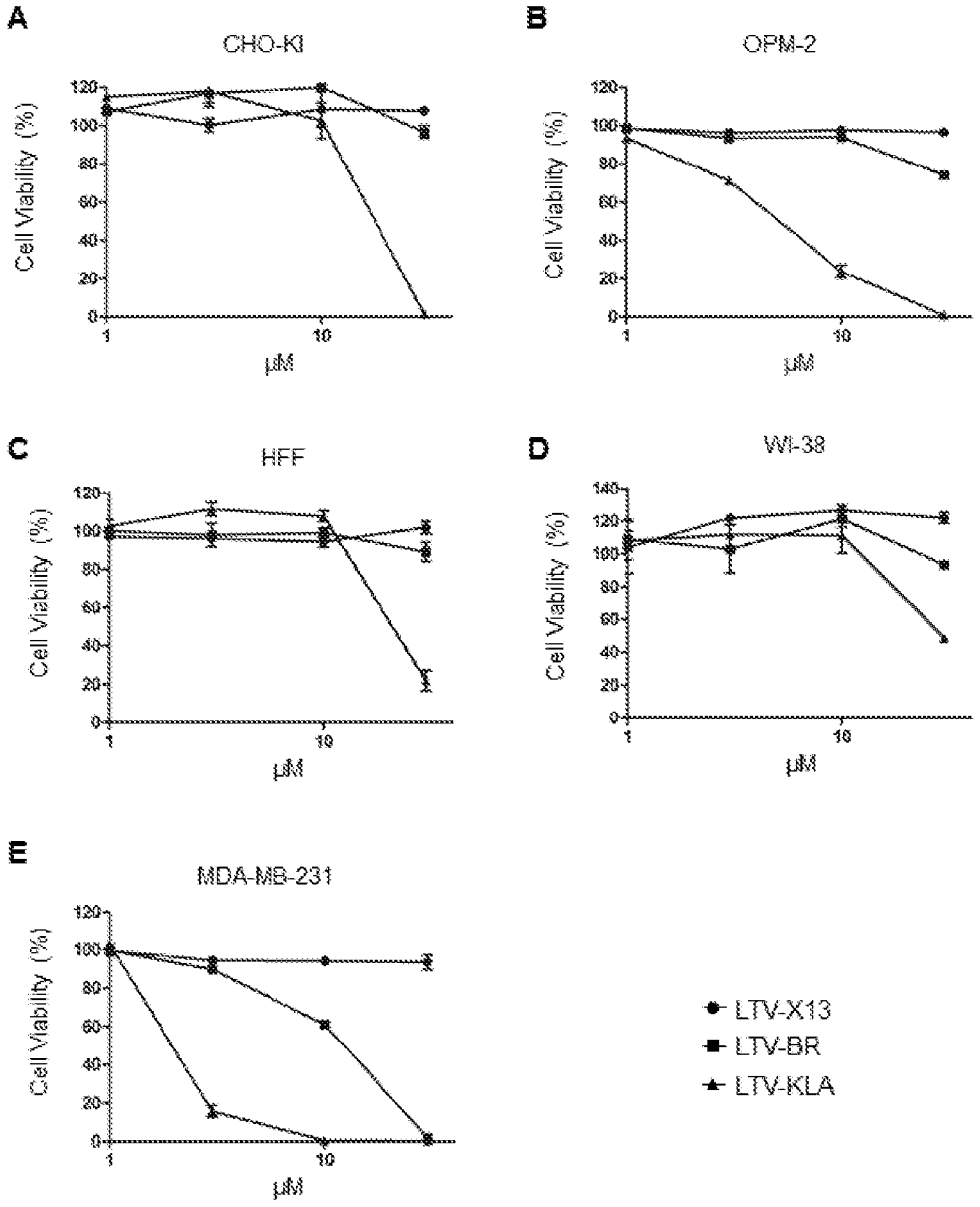

A
CHO-KI
B
OPM-2
C
HFF
D
WI-38
E
MDA-MB-231
Cell Viability (%)
μM
LTV-X13
LTV-BR
LTV-KLA

Fig. 9

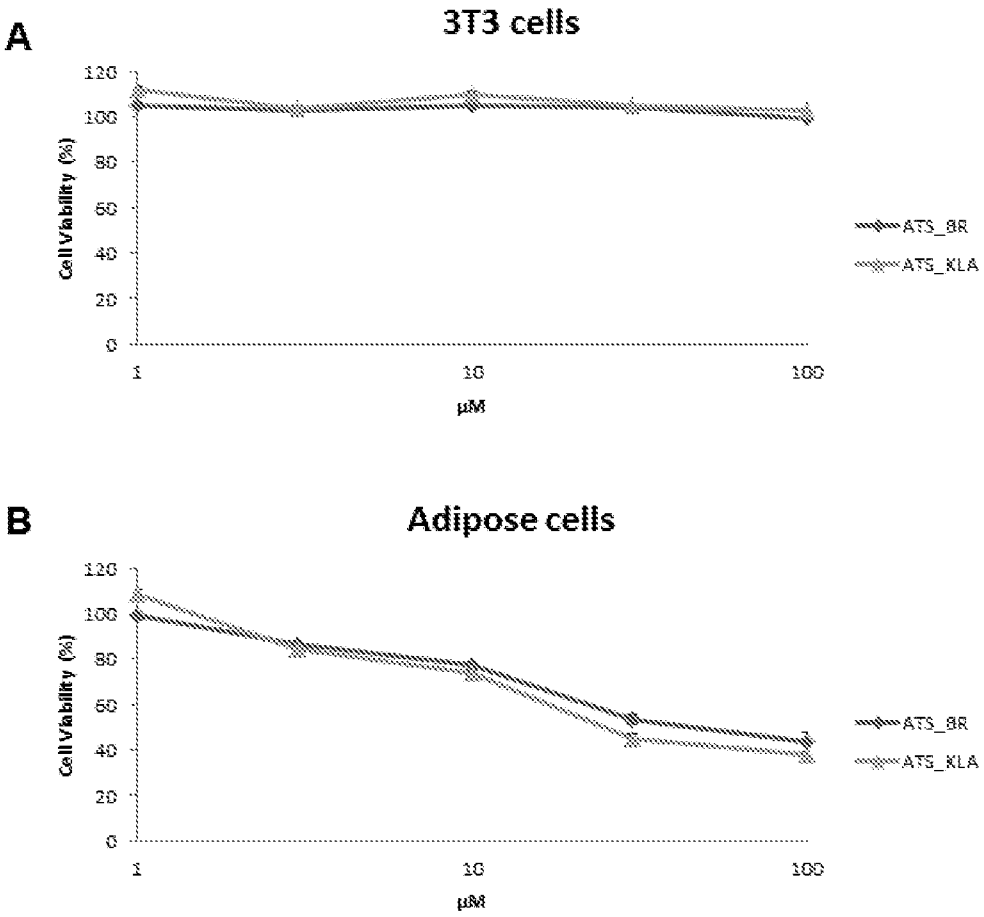

A
3T3 cells
Cell Viability (%)
120
100
80
60
40
20
0
1
10
100
μM
ATS_8R
ATS_KLA
B
Adipose cells
Cell Viability (%)
120
100
80
60
40
20
0
1
10
100
μM
ATS_8R
ATS_KLA

Fig. 10

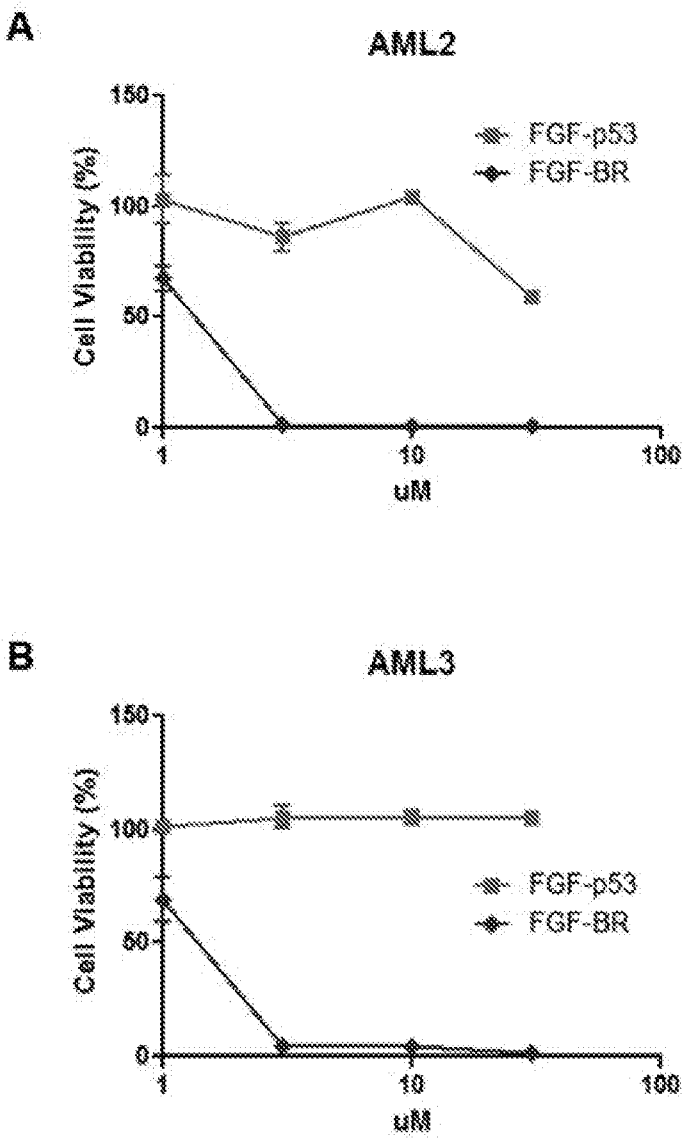

A
AML2
150
100
50
0
Cell Viability (%)
FGF-p53
FGF-BR
1
10
100
uM
B
AML3
150
100
50
0
Cell Viability (%)
FGF-p53
FGF-BR
1
10
100
uM

Fig. 11