

[19] Patents Registry
The Hong Kong Special Administrative Region
香港特別行政區
專利註冊處

[11] 1170419 B
EP 2512498 B1

[12]

STANDARD PATENT SPECIFICATION
標準專利說明書

[21] Application No. 申請編號
12111216.1

[51] Int.Cl.⁸ A61K C07K A61P A61Q

[22] Date of filing 提交日期
07.11.2012

[54] PEPTIDE PROTECTION AGAINST ULTRAVIOLET LIGHT TOXICITY 抗紫外光毒性之肽保護作用

[30] Priority 優先權

19.11.2009 US 61/262,790

[43] Date of publication of application 申請發表日期

01.03.2013

[45] Publication of the grant of the patent 批予專利的發表日期

05.02.2016

EP Application No. & Date 歐洲專利申請編號及日期

EP 10832158.9 18.11.2010

EP Publication No. & Date 歐洲專利申請發表編號及日期

EP 2512498 24.10.2012

Date of Grant in Designated Patent Office 指定專利當局批予專利日期

16.09.2015

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(11) **EP 2 512 498 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention
of the grant of the patent:

16.09.2015 Bulletin 2015/38

(21) Application number: **10832158.9**

(22) Date of filing: **18.11.2010**

(51) Int Cl.:

A61K 38/08 <small>(2006.01)</small>	A61K 8/64 <small>(2006.01)</small>
A61K 9/00 <small>(2006.01)</small>	C07K 7/06 <small>(2006.01)</small>
A61P 17/16 <small>(2006.01)</small>	A61Q 19/00 <small>(2006.01)</small>
A61Q 19/08 <small>(2006.01)</small>	A61Q 7/00 <small>(2006.01)</small>

(86) International application number:

PCT/US2010/057177

(87) International publication number:

WO 2011/063090 (26.05.2011 Gazette 2011/21)

(54) **PEPTIDE PROTECTION AGAINST ULTRAVIOLET LIGHT TOXICITY**

PEPTIDSCHUTZ GEGEN DIE TOXIZITÄT ULTRAVIOLETTEN LICHTS

PROTECTION PAR DES PEPTIDES CONTRE LA TOXICITÉ DU RAYONNEMENT ULTRAVIOLET

(84) Designated Contracting States:

**AL AT BE BG CH CY CZ DE DK EE ES FI FR GB
GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO
PL PT RO RS SE SI SK SM TR**

(30) Priority: **19.11.2009 US 262790 P**

(43) Date of publication of application:

24.10.2012 Bulletin 2012/43

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Description**FIELD OF THE INVENTION**

5 **[0001]** The invention relates to peptides having biological and therapeutic activity. Particularly, the invention relates to short peptides that protect against mutagen-induced cellular/tissue toxicity. For example, the inventive peptides protect against toxicity in the skin that occurs after UV light exposure. One function of the inventive peptides is to block the phosphorylation, and therefore activation, of certain cell cycle regulatory proteins. The disclosure is further related to methods of using the inventive peptides to reduce the level cellular/tissue toxicity following mutagen (e.g., UV light) exposure.

BACKGROUND OF THE INVENTION

15 **[0002]** When keratinocytes are exposed to DNA damaging elements (mutagens) such as ultraviolet (UV) radiation, cell cycle checkpoints are activated thereby blocking cell division. The arrest of the cell cycle in G2 phase after mutagen-induced damage allows time for DNA repair. However, if the checkpoint process is interrupted or inhibited, then the frequency of cancer-producing events (e.g., DNA mutations) is reduced. This has been demonstrated in vitro and in vivo through the application of caffeine to skin before and after UV irradiation (Lu et al., 2008, Cancer Res. 68:2523-2529; Heffernan et al., 2009, J. Invest. Dermatol. 129:1805-1815). The basis for this phenomenon is that caffeine inhibits the mitotic checkpoint pathway, allowing DNA-damaged cells to proceed to mitosis and die via apoptosis as their DNA is incapable of satisfactory replication. Allowing cell cycle progression despite DNA mutation/damage and the apoptotic cell death that occurs as a result prevents the fixation of mutagen-induced mutations in the affected tissue. This process thereby reduces the number of genetically altered cells that would otherwise have the potential to develop into cancerous lesions such as carcinoma.

25 **[0003]** The ability of caffeine to reduce the carcinogenic effects of UV light on skin has raised an interest in using this agent in skin care therapeutic and cosmetic skin care applications. However, the use of caffeine in skin care products is problematic given its lack of specificity. Aside from its salutary effects, caffeine can induce undesirable effects in skin (e.g., vasodilation, drying, etc.). Given these drawbacks, other approaches have been sought after for preventing the damaging effects of mutagens in skin and related tissues.

30 **[0004]** The use of short peptides for the development of skin care products is very popular due to their natural amino acid-based structure, specificity, lack of toxicity, and lack of side effects. These qualities render peptides as a suitable starting point for the development of novel chemopreventive agents for supplementation to skin care compositions. Peptides having chemopreventive and chemotherapeutic properties and that are applicable to protecting skin from the damaging effects of sunlight are described herein.

SUMMARY OF THE INVENTION

40 **[0005]** The invention is defined by the claims. An embodiment of the instant disclosure is directed to a method of reducing the activity of a cell cycle checkpoint kinase. Such a method can comprise exposing a checkpoint kinase to a peptide that is five to nine amino acid residues in length and comprises SEQ ID NO:13. The cell cycle checkpoint kinase in this and other embodiments may be in the activated state. Examples of peptides that can be applied in this or other methods described herein comprise or consist of SEQ ID NO:2, 3, 5, 6, 8, or 10.

45 **[0006]** Other examples of peptides that can be used in the methods described herein are those having a histidine residue that is directly contiguous with the SEQ ID NO:13 sequence. Other peptides can be amidated at the carboxy terminus thereof. Specific examples of such amidated peptides are those comprising or consisting of SEQ ID NO:2, 3, 5, 6, 8, or 10.

50 **[0007]** Checkpoint kinases targeted by the disclosed methods may comprise or consist of checkpoint kinase-1 (Chk1) or checkpoint kinase-2 (Chk2). The checkpoint kinase may be activated as a result of DNA damage in a cell. Such DNA damage may be incurred by a mutagenic agent. Examples of mutagenic agents are those that are capable of inducing DNA damage in skin (e.g., ultraviolet radiation). The disclosed methods can also comprise exposing a cdc25 (cell division cycle 25) phosphatase to a peptide described herein; cdc25c is an example of a cdc25c that can be targeted in the instant invention.

55 **[0008]** Another embodiment of the instant disclosure is directed to a method of treating the skin of a mammal. Such a method can comprise reducing the activity of a cell cycle checkpoint kinase (e.g., Chk2) in the skin by exposing the checkpoint kinase to a disclosed peptide. Another embodiment of the instant invention is directed to a treating the skin of a mammal by applying a disclosed peptide to the skin.

[0009] Another embodiment of the instant disclosure is directed to a peptide that is five to nine amino acid residues in length and comprises SEQ ID NO:13. Examples of such a peptide comprise or consist of SEQ ID NO:2, 3, 5, 6, 8, or

10. Other examples of peptides of the invention have a histidine residue that is directly contiguous with the SEQ ID NO:13 sequence. Other peptides can be amidated at the carboxy terminus thereof. Specific examples of such amidated peptides are those comprising or consisting of SEQ ID NO:2, 3, 5, 6, 8, or 10.

[0010] Another embodiment of the instant invention is directed a composition comprising a peptide of the instant invention and a pharmaceutically acceptable carrier. The composition can be in the form of an aerosol, emulsion, liquid, lotion, cream, paste, ointment, powder, or foam.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011]

FIG. 1 shows the amino acid sequences of certain inventive peptides (SEQ ID NOs:1-10) as they generally align with each other and SEQ ID NO:11 (human tyrosinase). The underlined residues in certain of the peptides (SEQ ID NOs:1-7) constitute conservative amino acid substitutions with respect to SEQ ID NO:11.

FIG. 2 shows the inhibition of checkpoint kinase 2 (Chk2) activity by staurosporine or certain inventive peptides. Control Chk2 activity is shown with the first bar. The peptides shown in the figure (SEQ ID NOs:2-6 and 8-10) are carboxy-terminal amidated (-CONH₂).

FIG. 3 shows the amino acid sequence of human Chk2 (SEQ ID NO:17). This sequence is the same as that disclosed at the U.S. National Center for Biotechnological Information (NCBI) website (or GenBank) under accession number AAH04207.

FIG. 4 shows the amino acid sequence of human Chk1 (SEQ ID NO:18). This sequence is the same as that disclosed at the NCBI website (or GenBank) under accession number AAC51736.

FIG. 5 shows the amino acid sequence of human cdc25c (SEQ ID NO:19). This sequence is the same as that disclosed at the NCBI website (or GenBank) under accession number AAR32098.

DETAILED DESCRIPTION OF THE INVENTION

[0012] The disclosed peptides (e.g., those listed in Table 1 and Figure 1) can comprise L- or D-amino acid enantiomers, either containing residues of one enantiomeric form or a combination of both forms. The peptides may be further augmented or modified as described in the following non-limiting examples, just so long as their primary amino acid sequences are unaltered; in this manner, the peptides consist of a certain amino acid sequence, but may comprise certain modifications. The carboxy-terminus of the peptides can be acidic (-COOH) or be amidated (e.g. -CONH₂, -CONHR, or -CONR₂). Amidation of the carboxy-terminus may render the disclosed peptides less susceptible to protease degradation and increase their solubility compared to their free acid forms, therefore providing heightened therapeutic potency. The peptides may also be lipidated, which may provide for enhanced skin penetration. One or more of the molecular bonds that link the amino acids of each peptide may be a non-peptide bond. Such non-peptide bonds include, but are not limited to, imido, ester hydrazine, semicarbazide and azo bonds.

Table 1

SEQ ID NO:	Sequence
1	DYHTLYQTHL
2	YHSLYQSHL
3	YHSIYQSHI
4	DFHSLFQSH
5	YHSLYESK
6	FHSIYQSH
7	FKSLYQS
8	HSLYQSH
9	HSLYQS
10	SLYQS
13	S-L/I-Y-Q/E-S
14	SIYQS

(continued)

SEQ ID NO:	Sequence
15	SLYES
16	SIYES

[0013] A variety of modifications can be made to the disclosed peptides as long as their primary amino acid sequences are retained. Some modifications may be used to increase the potency of the peptide, while other modifications may facilitate peptide handling. Peptide functional groups that may typically be modified include hydroxyl, amino, guanidinium, carboxyl, and amide groups. Typical, non-limiting reactions of these groups include the following: acetylation of hydroxyl groups by alkyl halides; esterification, amidation or hydrogenization (i.e., reduction to alcohol) of carboxyl groups; deamidation, acylation, alkylation, arylation of amino groups (e.g., primary amino group of the peptide or the amino group of lysine residues). SEQ ID NOs:1-10 and/or 13, for example, can be amidated at the carboxy terminus (-CONH₂).

[0014] The above discussion notwithstanding, the disclosed peptides can be designed to have certain amino acid alterations at one or more amino acid residue positions. For example, one, two, or three residues are changed to a conserved residue or residues. Amino acid conservation strategies are well known in the art; for example, it is well known that lysine, phenylalanine, isoleucine, threonine and glutamate can substitute for, respectively, histidine, tyrosine, leucine, serine and glutamine without largely affecting the structure and function of the original peptide. Optionally, the disclosed peptide may have one less amino acid compared to any one of SEQ ID NOs:1-10 or 13; or have two less amino acids (adjacent or non-adjacent). Variant forms of the disclosed peptide should be at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical with any one of SEQ ID NOs:1-10 or 13. Variant forms of the disclosed peptides should have at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 95% of the activity or function possessed by any one of SEQ ID NOs:1-10 or 13.

[0015] Examples of the activity or function possessed by SEQ ID NOs:1-10 and 13 are chemoprevention of cancer (e.g., carcinoma, sarcoma, melanoma, skin cancer), preventing development of a pre-cancerous cell population in tissue (e.g., skin) exposed to a mutagenic/carcinogenic agent (e.g., UV radiation), blocking cell cycle arrest and DNA repair following exposure of a cell (e.g., a skin cell, keratinocyte, melanocyte, or fibroblast) to a mutagenic/carcinogenic agent (e.g., UV radiation), blocking activation of cdc25c by a DNA damage repair pathway such as that orchestrated through a checkpoint kinase (e.g., checkpoint kinase-2 [Chk2]), blocking or reducing toxicity (such as in skin) related to or induced by exposure to a mutagen/carcinogen (e.g., UV radiation), blocking or reducing the negative effects of a mutagen/carcinogen on characteristics of healthy skin (e.g., tone, elasticity, hydration, coloration, firmness, smoothness). Chemoprevention (chemoprophylaxis) can refer to the use of agents such as the inventive peptides to reduce the risk of, or delay the development or recurrence of, neoplasia (e.g., cancer, pre-cancerous lesions, benign overgrowth lesions). Toxicity in the skin as induced by a mutagenic agent or any other agent (e.g., chemotherapeutic, irritant) can manifest in the form of erythema, alopecia (hair loss), photosensitivity (increased sensitivity to sunlight), recall reactions (e.g., effects of chemotherapy on site previously treated with radiation), acneiform (pimple-like) eruptions, skin necrosis, neutrophilic eccrine hidradenitis, eccrine squamous metaplasia, hyperpigmentation, nail changes, mucositis, sclerotic dermal reactions, vascular injury, xerosis, edema (swelling), urticaria, skin ageing (e.g., thinning, reduced elasticity, wrinkling, sagging, increased pigmentation [e.g., freckling, solar lentigo, guttate hypomelanosis], telangiectases, angioma, purpura, solar comedones, colloid milia, seborrheic keratoses), blistering, dermatitis, vegetating/fungating nodules (raised firm lumps), exudative plaques, vegetating or necrotic ulcer with pustules, scarring, panniculitis, ulceration, pain and/or burns. By "reducing," "inhibiting," "blocking," or "preventing" as referred to herein, it is meant that an inventive peptide brings down the occurrence, severity, magnitude, morbidity, or associated symptoms of a condition by at least about 7.5%, 10%, 12.5%, 15%, 17.5%, 20%, 22.5%, 25%, 27.5%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, or 100% compared to how the condition would normally exist without application of the peptide or composition comprising the peptide.

[0016] Though the disclosed peptides can be produced without the addition of other sequences, the option exists to incorporate the disclosed sequences within a larger sequence. For example, an disclosed peptide can have extra amino acid residues added to either or both of its N- and C-termini. An example would be a marker epitope such as Flag, which is commonly used to track proteins; marker epitopes well known in the art include myc, His, HA (hemagglutinin), for example. Thus, the present disclosure covers peptides that either consist of or comprise the inventive peptides. Although the disclosed peptides may be provided within a larger sequence (i.e., comprised within), the combination of the inventive peptide sequence with an additional sequence(s) should not constitute any protein or peptide sequence that is naturally expressed by an animal cell (e.g., human cell). Where language is used herein to describe that the peptide "consists" of a sequence, such a peptide, while being limited to a certain contiguous amino acid sequence, may comprise (i.e., be conjugated to) non-amino acid moieties or other peptides/proteins (such peptides/proteins would be conjugated to the

inventive peptide via a non-peptide bond).

[0017] The peptides of the current disclosure may be 5, 6, 7, 8, or 9 contiguous amino acids in length and comprise or consist of SEQ ID NO:13 (S-L/1-Y-Q/E-S). Non-limiting examples of such peptides are SEQ ID NOs:2, 3 and 5-10. Other non-limiting examples of such peptides are fragments of SEQ ID NOs:2, 3 and 5-8 that comprise SEQ ID NO:13. Following from the formula of SEQ ID NO:13, other peptides of the current disclosure may be 5, 6, 7, 8, or 9 contiguous amino acids in length and comprise or consist of SEQ ID NO:14 (S-I-Y-Q-S), SEQ ID NO:15 (S-L-Y-E-S), or SEQ ID NO:16 (S-I-Y-E-S). Other peptides of the current disclosure may comprise SEQ ID NO:13, wherein a histidine residue is directly contiguous with the SEQ ID NO:13 sequence; examples of such peptides are SEQ ID NOs:2, 3, 5, 6, 8 and 9. Short peptides containing SEQ ID NO:13 are all related with each other on a structural level, given the structural/chemical similarity between leucine and isoleucine (i.e., position 2 of SEQ ID NO:13) and between glutamine and glutamate (i.e., position 4 of SEQ ID NO:13). Other amino acids beside leucine and isoleucine may occupy position 2 of SEQ ID NO:13, such as alanine, valine, glycine, or methionine. Other amino acids beside glutamine and glutamate may occupy position 4 of SEQ ID NO:13, such as asparagine, aspartate, arginine, or lysine.

[0018] Alternatively, the peptides of the current disclosure may be 5, 6, 7, 8, or 9 contiguous amino acids in length and comprise or consist of SEQ ID NO:10 (S-L-Y-Q-S). Non-limiting examples of such peptides are SEQ ID NOs:2 and 7-9. Other non-limiting examples of such peptides are fragments of SEQ ID NOs:2, 7 and 8 that comprise SEQ ID NO:10. Other peptides of the current invention may comprise SEQ ID NO:10, wherein a histidine residue (H) is directly contiguous with the SEQ ID NO:10 sequence; examples of such peptides are SEQ ID NOs:2, 8 and 9.

[0019] All of the embodiments of the inventive peptides may be in the "isolated" state. For example, an "isolated" peptide is one that has been completely or partially purified. In some instances, the isolated peptide will be part of a greater composition, buffer system or reagent mix. In other circumstances, the isolated peptide may be purified to homogeneity. A composition may comprise the peptide at a level of at least about 50, 80, 90, or 95% (on a molar basis) of all the other macromolecular species that are also present therein. The inventive peptides may comprise heterologous combinations of components. Mixtures of the inventive peptides may be used in practicing the invention.

[0020] The inventive peptides may be conjugated to soluble or insoluble carrier molecules to modify their solubility properties as needed and to increase the local concentrations of peptides in targeted tissues. Examples of soluble carrier molecules include polymers of polyethylene glycol (PEG) and polyvinylpyrrolidone; examples of insoluble polymers include silicates, polystyrene, and cellulose. Peptides may also be micro-encapsulated to enhance their stability during and after therapeutic application; typically, polyester and PEG microspheres are used to encapsulate and stabilize the peptides.

[0021] Various methods of preparing microspheres for peptide encapsulation may be employed depending upon the hydrophilic or hydrophobic nature of the peptide composition to be encapsulated. Examples of protocols for such methods are found in Wang et al. (J. Control. Release 17:23, 1991) and U.S. Patent No. 4,324,683. *In vitro* peptide release studies may be performed to determine the relative availability of the peptide after incorporation into a microsphere. Microspheres (200 mg) are suspended in 2.5 mL phosphate-buffered saline (PBS, pH 7.2) and agitated at 37 °C and 100 rpm in an environmental incubator shaker (G-24, New Brunswick Scientific Co., Edison, N.J.). At specific sampling times (each day for the first 4 days and every other day thereafter) the buffer solution is completely removed and replaced with fresh PBS. The peptide content of the PBS is measured using the Bradford method or other suitable quantitative assay typically used for protein analysis.

[0022] The following procedures and parameters are provided for guidance purposes only and are all well known to those skilled in the art. All the disclosed peptides may be synthesized using standard Fmoc (9-fluorenylmethoxycarbonyl) solid-phase chemistry on an Advanced ChemTech Apex 396 Multiple Peptide Synthesizer. The Apex 396 is equipped with a 40-well reaction block for the production of up to 40 peptides simultaneously at a scale of 0.15 mmol. The peptides can be prepared as either amidated or free acid sequences using standard amino acids. The resin is first washed and pre-swelled with N,N-dimethyl formamide (DMF). The swelling time is one hour for Rink amide resins. The Fmoc protecting group is removed with 25% piperidine in DMF for 25 minutes, after which the piperidine is completely washed from the resin. To control racemization processes, the Fmoc amino acid monomers are pre-activated in an equimolar solution of 1-hydroxy-benzotriazole (HOBt) or 1-hydroxy-7-aza-benzotriazole (HOAt) in 0.5 M DMF. The amide couplings are carried out using O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) PyBop® or 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) as an activation agent and 2.5-5.0 fold molar excess of amino acid under basic conditions using a hindered base (diisopropylethylamine). The coupling times are 1-1.5 hours followed by a wash and recoupling to accomplish a double or triple couple before deprotection and continuation of the growing peptide chain. Coupling efficiency is monitored using the standard Kaiser test. Once peptide synthesis is completed on the resin, the final Fmoc group is removed as above and the sequences are left as the free base form.

[0023] Cleavage of the acid-labile linkage of the peptide to the resin is accomplished using 95% trifluoroacetic acid (TFA) and water with the appropriate scavengers added. After cleavage is allowed to proceed for about 30 minutes to one hour, the released peptides are immediately removed from the cleavage block and transferred to tubes for the removal of the TFA under reduced pressure. The peptides are then ready for purification and analysis via high performance

liquid chromatography (HPLC) using a reverse phase C18 column and mass spectrometry. Primary sequence confirmation and preparative purification are accomplished using an LC/MS/MS system (ABI API2000).

[0024] General to the above protocol, the peptides may be produced using any method known to those skilled in the art such as those disclosed in Merrifield (J Am Chem Soc. 85:2149, 1963); Carpino et al. (J Org Chem. 51:3732, 1986); Merrifield et al. (Anal Chem. 38:1905, 1966); or Kent et al. [High Yield Chemical Synthesis Of Biologically Active Peptides On An Automated Peptide Synthesizer Of Novel Design, IN: PEPTIDES 1984 (Ragnarsson, ed.) Almquist and Wiksell Int., Stockholm (Sweden), pp. 185-188]. The peptides can be produced by a machine capable of sequential addition of amino acids to a growing peptide chain. However, the peptides may also be manufactured using standard solution phase methodology, which can be amenable to large-scale production efforts.

[0025] Additional embodiments of the current invention are directed towards methods of using the above-described peptides, such as in formulations or as therapeutic agents. These methods may involve the use of a single peptide, or multiple peptides in combination (i.e., a mixture).

[0026] In certain instances, the inventive composition can be disposed within devices placed upon, in, or under the skin. Such devices include transdermal patches, implants, and injections which release the substances in such a manner as to contact the skin or hair follicle either by passive or active release mechanisms. The substance can be applied, for example, topically to the epidermis at regular intervals, such as once or twice daily, in a suitable vehicle and at an effective concentration. One or more injections to the skin offer another route for administering the inventive peptides to the skin or any other tissue.

[0027] The compositions used to deliver the peptides in the methods described herein can be in the form of an aerosol, emulsion, liquid, lotion, cream, paste, ointment, powder, foam, or other pharmaceutically acceptable formulation. Furthermore, the peptides can be delivered using less involved formulations such as deionized/distilled water, PBS or standard medical saline solutions. Generally, a pharmaceutically acceptable formulation would include any carrier suitable for use on human skin or mucosal surface. Such pharmaceutically acceptable carriers include ethanol, dimethyl sulfoxide, glycerol, silica, alumina, starch, and equivalent carriers and diluents. The formulation may optionally have cosmetic appeal, and/or contain other agents such as retinoids or other peptides that can act as adjuvants for the therapeutic action of the inventive peptides. Antibiotics can also be added to the formulation in order to ward off infection, thereby permitting maximal healing processes to occur. The concentration of the peptide in the composition can be about 0.1 $\mu\text{g/mL}$ to about 50 $\mu\text{g/mL}$ or about 0.1 $\mu\text{g/mL}$ to about 20 $\mu\text{g/mL}$; however, the ultimate concentration employed may vary outside these ranges, depending on the nature of the target tissue, the bio-activity of the inventive peptide and the use of any adjuvant or technique to obtain enhanced composition absorption. Such determinations are well within the normal skill in the art. For example, the concentration of the peptide(s) used in practicing the instant invention can be about 0.1, 1, 2, 5, 10, 15, 20, 25, 50, 75, 100, 200, 500, or 1000 $\mu\text{g/mL}$.

[0028] The administration of the inventive peptides and associated compositions may be made to humans and animals, including all mammals (e.g., pigs, cows, horses, sheep, goats, mice, rats, cats, dogs, ferrets, primates). Application may also be made in combination with typical and/or experimental materials such as tissue grafts, tissue culture products, oxygen and dressings. In general, the composition can be administered topically, orally, transdermally, systemically, or by any other method known to those of skill in the art to be useful to deliver the inventive peptides to the target tissue. Compositions may also be applied in an *in vitro* or *ex vivo* manner, either to cells or patient grafts growing in culture, for example.

[0029] Due to their small size, the peptides are expected to be able to gain by themselves a level of permeability through the skin; however, certain techniques may be used to amplify this movement. For example, lipophilic (non-polar) groups can be added to the peptides, or the peptides can be delivered to the skin in a lipophilic excipient, in order to enhance peptide accessibility to the stratum corneum to allow translocation to the lower epidermal layers. In this manner such lipophilic modifications may be considered as having a pro-drug effect. Permeation enhancers such as known solvents and surfactants may be used in the excipient to allow better peptide absorption. Special techniques that are anticipated to be useful in enhancing peptide access to the targeted tissue/injury include, injection regimens, iontophoresis, electrophoresis and ultrasound. An iontophoretic device consists of two electrodes immersed in an electrolyte solution and placed on the skin. When an electric current is applied across the electrodes, an electric field is created across the stratum corneum that drives the delivery of the peptides. Electroporation involves the application of high-voltage electric pulses to increase the permeation through lipid bilayers. This differs from iontophoresis in the duration and intensity of the application of electrical current (iontophoresis uses a relatively constant low-voltage electric field). The high-voltage electric pulse of electroporation is believed to induce a reversible formation of hydrophilic pores in the lipid lamellae membranes that can provide a high degree of permeation enhancement. Ultrasound applies sound waves having a frequency greater than 16 kHz to the skin, which causes compression and expansion of the tissue through which the sound waves travel. The resulting pressure variations cause a number of processes (e.g., cavitation, mixing, increase in temperature) that may enhance permeation of the peptides.

[0030] The instant invention can comprise one or more protease inhibitors. A protease inhibitor can be selected to

specifically target proteases that would be expected to degrade the selected bioactive peptide; such a selection would be determined based on the length and/or sequence of the bioactive peptide. However, protease inhibitors need not necessarily be selected in any specific manner; for example, a protease inhibitor cocktail, which contains two or more inhibitors, can be employed in the instant invention. With certain embodiments of the invention, the protease inhibitor is not one that is specific to inhibiting a virus. The following types of protease inhibitors can be incorporated in the invention: serine protease inhibitors, cysteine protease inhibitors, aspartate protease inhibitors, metalloproteinase inhibitors, thiol protease inhibitors and threonine protease inhibitors

[0031] Protease inhibitors are well known in the art. Non-limiting examples of protease inhibitors that can be incorporated in the present invention include acetyl-pepstatin, AEBSF (4-[2-Aminoethyl] benzenesulfonyl fluoride) hydrochloride, ALLM (N-Acetyl-Leu-Leu-Met), ALLN (N-Acetyl-Leu-Leu-Nle-CHO), amastatin (*Streptomyces* sp.), ϵ -amino-*n*-caproic acid, aminopeptidase N inhibitor, α_1 -antichymotrypsin, antipain (hydrochloride or dihydrochloride), α_2 -antiplasmin, antithrombin III, α_1 -antitrypsin, *p*-APMSF hydrochloride, aprotinin (e.g., from bovine lung), ATBI (an 11-residue peptide), benzamidine hydrochloride, bestatin, bestatin methyl ester, calpastatin, calpeptin, carboxypeptidase inhibitor, caspase inhibitor, cathepsin B inhibitor II, cathepsin G inhibitor I, cathepsin inhibitor II, cathepsin inhibitor III, cathepsin inhibitor I, cathepsin K inhibitor I, cathepsin K inhibitor II, cathepsin K inhibitor III, cathepsin L inhibitor I, cathepsin L inhibitor II, cathepsin L inhibitor IV, cathepsin L inhibitor V, cathepsin L inhibitor VI, cathepsin S inhibitor, cathepsin/subtilisin inhibitor, chymostatin, chymotrypsin inhibitor I, cystatin, 1,5-dansyl-glu-gly-arg chloromethyl ketone dihydrochloride, 3,4-dichloroisocoumarin, diisopropylfluorophosphate, dipeptidylpeptidase II inhibitor, dipeptidylpeptidase IV inhibitor I, dipeptidylpeptidase IV inhibitor II, E-64 protease inhibitor, ecotin, EDTA disodium salt dihydrate, EDTA tetrasodium salt, EGTA, elastase inhibitor I, elastase inhibitor II, elastase inhibitor III, elastatinal, 6-amidino-2-naphthyl-4-guanidinobenzoate dimethanesulfonate, glu-gly-arg-chloromethyl ketone, 2-guanidinoethylmercaptosuccinic acid, hexadecylsulfonyl fluoride, α -iodoacetamide, kininogen, leuhistin, leupeptin hemisulfate, α_2 -macroglobulin, DL-2-mercaptomethyl-3-guanidinoethylthiopropionic acid, pepstatin A, phenylmethylsulfonyl fluoride, phosphoramidon Disodium Salt, PPack II trifluoroacetate salt, PPack dihydrochloride, prolyl endopeptidase inhibitor II, Na-tosyl-lys chloromethyl ketone hydrochloride, Na-tosyl-phe chloromethyl ketone, tripeptidylpeptidase II inhibitor, trypsin inhibitor (from corn or soybean), D-val-phe-lys chloromethyl ketone dihydrochloride, 1,3-di-(N-carboxybenzoyl-L-leucyl-L-leucyl)amino acetone, α -phenanthroline, ursolic acid (e.g., Rosemary extract), tranexamic acid (4-[aminomethyl]cyclohexane-1-carboxylic acid) (clinically marketed as Cyklokapron in the U.S. and as Transamin in Asia), Fmoc-Lys(Boc), Fmoc-Arg(Pmc), benzoyl-Arg-nitroanilide, benzoyl-Arg-naphthylamide, and α -2-macroglobuline.

[0032] The protease inhibitor used in the invention may be a peptide or protein, such as an enzyme. Non-limiting examples of such inhibitors are the serpins, which include alpha-1-antitrypsin, complement 1-inhibitor, antithrombin, alpha-1-antichymotrypsin, plasminogen activator inhibitor 1, and neuroserpin.

[0033] Components that are typically incorporated into skin care preparations are well known in the art. Beside the bioactive peptide component, the instant invention can contain other active agents such as niacinamide, phytantriol, farnesol, bisabolol and salicylic acid. It is expected that certain additional active agents will act synergistically with the bioactive peptide component, or will enhance the shelf-life of the formulation.

[0034] Where the composition is to be in contact with animal or human skin, additional components should be chosen that are suitable for application to keratinous tissue (i.e., stable, low toxicity, hypoallergenic). The CTFA Cosmetic Ingredient Handbook, Second Edition (1992), describes a wide variety of non-limiting cosmetic and pharmaceutical ingredients commonly used in the skin care industry that are suitable for use in the compositions of the present invention. Examples of these ingredients include: abrasives, absorbents, aesthetic components such as fragrances, pigments, colorings/colorants, essential oils, skin sensates, astringents, etc. (e.g., clove oil, menthol, camphor, eucalyptus oil, eugenol, menthyl lactate, witch hazel distillate), anti-acne agents (e.g., resorcinol, sulfur, salicylic acid, benzoyl peroxide, erythromycin, zinc), anti-caking agents, antifoaming agents, antimicrobial agents (e.g., iodopropyl butylcarbamate), antioxidants, binders, biological additives, buffering agents, bulking agents, chelating agents, chemical additives, denaturants, external analgesics, polymers (e.g., copolymer of eicosene and vinyl pyrrolidone), opacifying agents, pH adjusters, propellants, reducing agents, sequestrants, skin bleaching and lightening agents (e.g., hydroquinone, kojic acid, ascorbic acid [vitamin C], magnesium ascorbyl phosphate, ascorbyl glucosamine), skin-conditioning agents (e.g., humectants, including miscellaneous and occlusive), skin soothing and/or healing agents (e.g., panthenol and derivatives [e.g., ethyl panthenol], aloe vera, pantothenic acid and its derivatives, allantoin, bisabolol, dipotassium glycyrrhizinate), thickeners, particulate materials, structuring agents and vitamins. Many of these agents are described in detail in U.S. Patent No. 6,492,326, specifically with respect to the various ingredient descriptions.

[0035] The compositions of the present invention may contain a particulate material such as a metallic oxide. These particulates can be coated or uncoated, charged or uncharged. Non-limiting examples of particulate materials useful for preparing the instant invention include bismuth oxychloride, iron oxide, mica, mica treated with barium sulfate and TiO₂, silica, nylon, polyethylene, talc, styrene, polypropylene, ethylene/acrylic acid copolymer, sericite, aluminum oxide, silicone resin, barium sulfate, calcium carbonate, cellulose acetate, titanium dioxide, polymethyl methacrylate, and mixtures thereof. Inorganic particulate materials such as TiO₂, ZnO (zinc oxide), or ZrO₂ are commercially available from a number

of sources. Particulate materials can be present in the composition at levels of from 0.01% to 2% by weight, or from 0.05% to 1.5% by weight, or from 0.1 % to 1 % by weight (all measures approximate).

[0036] The compositions of the present invention may contain a conditioning agent selected from humectants, moisturizers, or skin conditioners. A variety of these materials can be employed and each can be present at a level of from about 0.01% to 20%, or from about 0.1% to 10%, or from about 0.5% to 7% by weight of the composition (all measures approximate). These materials include, but are not limited to, guanidine; urea; glycolic acid and glycolate salts (e.g. ammonium and quaternary alkyl ammonium); salicylic acid; lactic acid and lactate salts (e.g., ammonium and quaternary alkyl ammonium); aloe vera in any of its variety of forms (e.g., aloe vera gel); polyhydroxy alcohols such as sorbitol, mannitol, xylitol, erythritol, glycerol, hexanetriol, butanetriol, propylene glycol, butylene glycol and hexylene glycol; polyethylene glycols; sugars (e.g., melibiose) and starches; sugar and starch derivatives (e.g., alkoxylated glucose, fructose, glucosamine); hyaluronic acid; lactamide monoethanolamine; acetamide monoethanolamine; panthenol; allantoin; petroleum jelly; and mixtures thereof.

[0037] The compositions of the present invention can contain a structuring agent, which can be used for preparing a oil-in-water emulsion. Without being limited by any theory, it is believed that the structuring agent assists in providing rheological characteristics to the composition which contribute to the stability of the composition. For example, the structuring agent tends to assist in the formation of liquid crystalline gel network structures. The structuring agent may also function as an emulsifier or surfactant. The instant invention may contain from about 0.1% to 20%, from about 0.1% to 10%, or from about 0.5% to 9% of one or more structuring agents by weight of the composition (all measures approximate).

[0038] Structuring agents than can be incorporated in the present invention are selected from stearic acid, palmitic acid, stearyl alcohol, cetyl alcohol, behenyl alcohol, the polyethylene glycol ether of stearyl alcohol having an average of about 1 to about 5 ethylene oxide units, the polyethylene glycol ether of cetyl alcohol having an average of about 1 to about 5 ethylene oxide units, and mixtures thereof. Other structuring agents that can be used in the present invention are selected from stearyl alcohol, cetyl alcohol, behenyl alcohol, the polyethylene glycol ether of stearyl alcohol having an average of about 2 ethylene oxide units (steareth-2), the polyethylene glycol ether of cetyl alcohol having an average of about 2 ethylene oxide units, and mixtures thereof.

[0039] Additional features, modes of production and use of the inventive peptides are described, for example, in U.S. Patent Nos. 6,974,799 and 5,492,894.

Methods

[0040] The instant disclosure can also drawn to a method of inhibiting or preventing neoplastic growth in a tissue of a mammal by administering peptides described herein. While not being held to any particular mechanism of action, the anti-neoplastic activity of the disclosed peptides is associated with the ability to inhibit the activity of cell cycle checkpoint kinases such as checkpoint kinase-2 (Chk2). Inhibiting Chk proteins, which are activated after a cell suffers DNA damage, prevents cells from entering cell cycle arrest, during which time DNA repair occurs. While DNA repair can return sequences back to their original state (i.e., non-mutated), it is usually insufficient to do so with large genetic insults. With the latter situation, the repair process oftentimes fixes (makes permanent) genetic alterations that can contribute to oncogenic processes (e.g., activation of an oncogene, inactivation of a tumor suppressor or gatekeeper gene). By blocking or reducing cell cycle arrest through the inhibition of Chk protein activity, cells can continue to the mitotic phase during which, due to the disruptive effects of certain DNA damage events (e.g., DNA double-strand breaks) on the division process, apoptosis is induced. DNA damage can also be referred to as genotoxic stress. Apoptosis therefore clears out cells carrying deleterious genetic changes; such cells would potentially survive and serve as precursors for neoplastic development if cell cycle arrest is permitted to fully activate thereby allowing for DNA "repair" to occur. In this manner, the instant invention can be used to reduce the accumulation of mutations in a tissue due to the effects of DNA-damaging agents.

[0041] The neoplastic growth prevented, inhibited, or treated by the inventive peptides can be benign, pre-cancerous, or cancerous (i.e., malignant). For this reason the disclosed peptides may be considered to have chemopreventive, chemoprophylactic, anti-cancer, anti-neoplastic, anti-carcinogenic, or chemotherapeutic activity. With respect to preventing or inhibiting a neoplastic growth or lesion in a tissue, one or more peptides can be administered to the tissue that otherwise shows no obvious (macroscopic) signs of neoplasia. For example, the peptides can be administered to a healthy tissue before the occurrence of a mutagenic insult (e.g., within 1, 2, 3, 4, 8, 12, 16, 20, 24, 48, 72, 96, or 120 hours pre-insult) (e.g., applied topically to the skin before sun exposure), or shortly after suffering a mutagenic insult (e.g., within 1, 2, 3, 4, 8, 12, 16, 20, 24, 48, 72, 96, or 120 hours post-insult) (e.g., applied topically to the skin before sun exposure). With regard to skin care, the peptides can be applied in either a sunscreen or after-sun preparation. The peptides can also be used to prevent or inhibit the increase in mutations in a tissue, whether it is completely healthy or contains small benign and/or pre-cancerous lesions. Such use can prevent or inhibit the transition of a benign or pre-cancerous lesion into a cancerous/malignant lesion (i.e., prevent or inhibit tumorigenesis), and can thus be said to treat

the benign or pre-cancerous lesion. In another aspect, the instant invention can be used to prevent or inhibit hyperplasia in the skin or other tissue.

[0042] Examples of benign and/or pre-cancerous neoplastic skin lesions that are prevented or inhibited in their occurrence by the instant invention, or treated, are dermatofibromas, epidermal cysts, hemangiomas, port-wine stains, lymphangiomas, pyogenic granulomas, spider angiomas (nevus araneus), keloids, keratoacanthomas, lipomas, moles, dysplastic nevus, seborrheic keratoses, skin tags, sebaceous hyperplasia, psoriasis and actinic keratosis (solar keratosis). Examples of malignant skin lesions/tumors in skin (i.e., skin cancer) that are prevented or inhibited in their occurrence by the instant invention, or treated, are basal cell carcinoma (e.g., nodular basal cell carcinoma, cystic basal cell carcinoma, cicatricial basal cell carcinoma, infiltrative basal cell carcinoma, micronodular basal cell carcinoma, superficial basal cell carcinoma, pigmented basal cell carcinoma, rodent ulcer [Jacobi ulcer], fibroepithelioma of Pinkus, polypoid basal cell carcinoma, pore-like basal cell carcinoma, aberrant basal cell carcinoma), squamous cell carcinoma (e.g., adenoid squamous cell carcinoma [pseudoglandular squamous cell carcinoma], clear cell squamous cell carcinoma, spindle cell squamous cell carcinoma, signet-ring cell squamous cell carcinoma, basaloid squamous cell carcinoma, verrucous carcinoma, keratoacanthoma), melanoma (e.g., lentigo maligna melanoma, superficially spreading melanoma, acral lentiginous melanoma, mucosal melanoma, nodular melanoma, polypoid melanoma, desmoplastic melanoma, amelanotic melanoma, soft-tissue melanoma, uveal melanoma), dermatofibrosarcoma protuberans, Merkel cell carcinoma, Kaposi's sarcoma, keratoacanthoma, spindle cell tumors, sebaceous carcinomas, microcystic adnexal carcinoma, atypical fibroxanthoma, leiomyosarcoma, and angiosarcoma.

[0043] Accordingly, the instant invention prevents, inhibits, or treats the development and/or progression of neoplasms in the skin for which the affected cell (i.e., cell type giving rise to the neoplasm) is an epithelial cell, a mesenchymal cell, a keratinocyte, a fibroblast, a melanocyte, a skin stem cell, or a skin progenitor cell. The tissues of the skin targeted by the instant invention are the epidermis, which comprises the stratum basale layer, stratum spinosum layer, stratum granulosum layer, stratum lucidum layer and stratum corneum layer; the dermis, which comprises the papillary and reticular layers; and the subcutaneous tissue, which comprises fat tissue, connective tissue, nerve tissue and blood vessels. All of the aforementioned tissues, layers and cell types are targeted by the instant invention.

[0044] Other examples of benign and/or pre-cancerous neoplastic lesions or growth profiles that are prevented or inhibited in their occurrence by the instant invention, or treated, are hyperplasia, dysplasia and metaplasia. Other examples of cancerous neoplastic lesions that are prevented, inhibited, or treated by the instant invention are lung cancer, bone cancer, pancreatic cancer, gastric, head or neck cancer, uterine cancer, ovarian cancer, gynecological cancer, rectal cancer, stomach cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, endometrial cancer, cervical cancer, vaginal cancer, vulvar cancer, Hodgkin's Disease, esophageal cancer, cancer of the small intestine, colon cancer, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, urethral cancer, penile cancer, prostate cancer, chronic or acute leukemia, lymphocytic lymphomas, bladder cancer, kidney or ureter cancer, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors, brain cancer, pituitary adenoma, hemangioma, glioma, or blastoma.

[0045] The instant invention can be practiced as a cosmetic or in the cosmetic treatment of skin. Accordingly, the instant invention acts to maintain normal, healthy skin traits, such as tone, elasticity, hydration, coloration, firmness and smoothness. All of these qualities can degrade with an increase in the amount of damaged cells in the skin, such damaged cells resulting from the effects of mutagens and/or the accumulation of mutations in the skin. Accordingly, the instant invention can prevent, inhibit or treat the effects of aging of the skin; photoaging is one example, where the skin ages partly as a function of degree of UV radiation exposure. The effects of photoaging targeted by the invention are wrinkles, mottling and/or hyperpigmentation, rough or leathery skin, droopiness/sagging, yellowing, dryness and various neoplasms, for example.

[0046] Tissues that can be targeted in practicing the instant invention are the skin and associated mucosal tissues of the skin. An associated mucosal tissue of the skin is any tissue organized in a manner similar to the skin, contains epithelial cells, and is directly continuous with the skin. Examples of such tissues are oral, nasopharyngeal, aural, anal and urogenital surfaces, as well as the palpebral conjunctiva of the eye. Other tissues that can be targeted in practicing the instant invention are those derived from the ectoderm, mesoderm and endoderm, or comprise epithelial cells, mesenchymal cells (e.g., fibroblasts), muscle cells, or nerve cells (e.g., neurons). Other organs, organ systems and tissues targeted by the invention are, for example, the circulatory system (e.g., heart, blood, blood vessels), digestive system (e.g., salivary glands, esophagus, stomach, liver, gallbladder, pancreas, small and large intestines, rectum), endocrine system (e.g., hypothalamus, pituitary gland, pineal gland, thyroid, parathyroids, adrenal glands), integumentary system (e.g., skin, hair, nails), lymphatic system (e.g., lymph nodes and vessels), immune system (e.g., tonsils, adenoids, thymus, spleen), muscular system (e.g., cardiac muscle, smooth muscle, skeletal muscle), nervous system (e.g., brain, spinal cord, peripheral nerves, nerves), reproductive system (e.g., ovaries, fallopian tubes, uterus, vagina, mammary glands, testes, vas deferens, seminal vesicles, prostate, penis), respiratory system (e.g., pharynx, larynx, trachea, bronchi, lungs, diaphragm), skeletal system (e.g., bones, cartilage, ligaments, tendons), and excretory system (e.g.,

kidneys, ureters, bladder, urethra).

[0047] As used in practicing the instant invention, the disclosed peptides can act to limit the occurrence of neoplasia (e.g., hyperplasia) by reducing the accumulation of mutations in a tissue resulting from exposure to mutagens of endogenous or ectopic (environmental) origin. The mutagen exposure time may be either acute or chronic. In general, the instant invention can be geared toward blocking mutation accumulation as a result of ectopic mutagen/carcinogen exposure. Examples of ectopic mutagens as discussed herein (i.e., those that originate from outside an animal from the surrounding environment) are ultraviolet (UV) radiation (i.e., UV light) (UV-A, UV-B, UV-C), ionizing radiation (e.g., X-rays, gamma rays, alpha particles), base analogs (e.g., 5-bromouracil), deaminating agents (e.g., nitrous acid [nitrite]), nitroamines, intercalating agents (e.g., ethidium bromide, proflavine, daunomycin, doxorubicin [adriamycin], thalidomide, dactinomycin, aflatoxins, acridine), alkylating agents (e.g., ethylnitrosourea), bromine, heat, sodium azide, psoralen, benzene, benzo-pyrenes, arsenic, asbestos, cadmium, chromium, ethylene oxide, nickel, radon, vinyl chloride, lead and viruses. Examples of endogenous mutagens are 5-methylcytosine, reactive oxygen species (E.G., nitric oxide, superoxide) and transposons. The instant invention is also directed to blocking the accumulation of mutations in a tissue that can result from application of certain irritants. For example, irritants can spur conditions of chronic inflammation, which can lead to cancer formation in various tissues.

[0048] Other chemopreventive and/or anticancer agents may be administered with the disclosed peptides in practicing the instant invention. Such administration may include a disclosed peptide and another chemopreventive agent together in the same composition, or involve a scheme where the peptide and agent are applied at different time points during the treatment/prevention regimen. Using both a disclosed peptide and another chemopreventive agent can create synergistic chemopreventive activity. Examples of chemopreventive and/or anticancer agents that can be used in conjunction with the present invention are phytochemicals, caffeine, caffeic acid, genistein, resveratrol, diallyl sulfide, S-allyl cysteine, allicin, lycopene, capsaicin, curcumin, 6-gingerol, ellagic acid, ursolic acid, silymarin, anethol, catechins, emodin, sulfuraphane, eugenol, isoeugenol, beta-carotene, oleandarin, polyphenols, indoles (e.g., di-indolylmethane, indole-3-carbinol), isothiocyanates (e.g., phenethylisothio-cyanate), non-steroidal anti-inflammatory agents (NSAIDS) (e.g., celecoxib, ibuprofen, sulindac, aspirin), PPAR-gamma agonists (e.g., pioglitazone, rosiglitazone), resiquimod, imiquimod, retinoids (e.g., all-trans-retinoic acid, 9-cis- or 13-cis-retinoic acid, 4-hydroxyretinamide, bexarotene, tararotene, selenium, soy isoflavones, statins (e.g., atorvastatin), sulfur containing antioxidants and vitamin D analogs (e.g., ergocalciferol, cholecalciferol).

[0049] One aim for inflicting DNA damage in cancer cells using certain chemotherapeutics or other agents (e.g., gamma-irradiation) is to induce cancer cells to undergo apoptosis thereby effecting tumor regression. However, induction of cell cycle arrest in response to DNA damage can depress this desired outcome. Therefore, another aspect of the instant invention is using the disclosed peptides in combination with a DNA-damaging agent to treat cancer (e.g., a skin cancer) or any other type of neoplasm. A basis for this method is the ability of the disclosed peptides to sensitize DNA-damaged cells to apoptosis (i.e., stimulate cells to undergo apoptosis instead of cell cycle arrest, which allows cancer cell survival) as discussed below. DNA-damaging agents that can be used in practicing the instant invention are, for example, alkylating agents, cisplatin, carboplatin, oxaliplatin, mechlorethamine, cyclophosphamide, chlorambucil, nitrogen mustards, mechlorethamine, ifosfamide, melphalan, nitrosoureas, streptozocin, carmustine (BCNU), lomustine, alkyl sulfonates, busulfan, bendamustine, triazines, dacarbazine (DTIC), temozolomide, ethylenimines, thiotepe, altretamine, hexamethylmelamine, antimetabolites, 6-mercaptopurine, dacarbazine, fludarabine, 5-fluorouracil (5-FU), capecitabine, methotrexate, gemcitabine, cytarabine, pemetrexed, arabinosylcytosine, decitabine, anti-tumor antibiotics, anthracyclines, daunorubicin, doxorubicin (e.g., Adriamycin®), epirubicin, idarubicin, actinomycin-D, bleomycin, mitomycin-C, neocarzinostatin, mitoxantrone, topoisomerase inhibitors, topoisomerase I inhibitors, topotecan, irinotecan, topoisomerase II inhibitors, etoposide, teniposide, plant alkaloids, taxanes, paclitaxel, docetaxel, epothilones, ixabepilone, vinca alkaloids, vinblastine, vincristine, vinorelbine, estramustine, radiation, gamma rays, X-rays and UV radiation. One or more of these agents may be applied to the lesion in combination with a disclosed peptide, or alternatively, a scheme can be practiced where the peptide and DNA-damaging agent are applied at different time points during the treatment regimen.

[0050] An aspect of the instant invention is the inhibition of checkpoint kinase (Chk) activity. For example, the invention is directed to inhibiting Chk2 that has been activated by DNA damage in a mammalian cell such as a skin cell. Such DNA damage and/or Chk activity may, for example, be induced in a cell via exposure to UV radiation or any other mutagen present in the environment. DNA damage induced by UV light generally comprises cyclobutane pyrimidine dimers and 6-4 pyrimidine-pyrimidone products. While not being held to any particular theory or mechanism, Chk is inhibited by the ability of the disclosed peptides to block or downregulate/down-modulate its inhibitory kinase activity toward cdc25 (cell division cycle 25) phosphatases such as cdc25c. Under normal cell conditions (little or no DNA damage), cdc25c sets off a signal cascade leading to mitosis, but under DNA damage conditions, activated Chk inhibits cdc25c. Therefore, blocking Chk activity increases the chances that cdc25c will be capable of signaling for mitosis, even under conditions of DNA damage which would normally (i.e., when Chk is not inhibited) downregulate cdc25c. DNA-damaged cells that are allowed entry to mitosis are later cleared by apoptosis, given the incompatibility of DNA damage

with normal mitotic processes. Accordingly, an aspect of the instant invention is directed to inducing the clearance of DNA-damaged cells via apoptosis. Another aspect of the instant invention is directed to inhibiting cell cycle arrest that normally occurs in response to DNA damage.

[0051] Examples of the Chk enzymes targeted by the instant invention are Chk1 and Chk2, both of which are well known in the art. These serine/threonine kinases are structurally and functionally conserved across eukaryotic species, the human versions of which phosphorylate human cdc25c phosphatase at serine-216 (or the equivalent serine residue, depending on where the Chk-targeted sequence is within a cdc25c variant [e.g., splice variant]). A method of using the inventive peptides to inhibit Chk1- and/or Chk2-mediated phosphorylation of cdc25c (e.g., at the serine-216 position or equivalent serine residue) is part of the instant invention. The instant invention can target the Chk proteins as they naturally exist in cells *in vivo*.

[0052] Activated Chk enzymes (such as they exist in response to DNA damage) can be activated by being phosphorylated by upstream factors (e.g., ATM [ataxia telangiectasia-mutated protein], ATR [ATM-RAD3-related protein]) in DNA damage-sensing pathways or via autophosphorylation. Non-activated forms of Chk may have little or no kinase activity compared to activated forms of Chk. Activated Chk enzymes can phosphorylate cdc25c, for example. Example Chk2 phosphorylation sites that can activate this enzyme are at the threonine-26, serine-50, threonine-68, threonine-383 and/or threonine-387 residue positions (or equivalent positions thereof within a Chk2 variant [e.g., splice variant]). Example Chk1 phosphorylation sites that can activate this enzyme are at the serine-286, serine-301, serine-317 and/or serine-345 residue positions (or equivalent positions thereof within a Chk1 variant [e.g., splice variant]).

[0053] Chk2, which is encoded by the CHEK2 gene in humans, has an amino acid sequence as shown in Figure 3 (SEQ ID NO:17). Human Chk2 is also known in the art as "CHK2 checkpoint homolog (*S. pombe*)," CDS1, and Rad53. SEQ ID NO:17 is presented for reference purposes only, since human Chk2 and other mammalian Chk2 protein sequences are known. For example, the U.S. National Center for Biotechnological Information (NCBI) website (or GenBank) discloses human Chk2 amino acid sequences under accession numbers AAH04207, BAB17231, NP_009125, NP_001005735, NP_665861, O96017, AAS58460, AAD48504, AAC83693, EAW59757, EAW59756, EAW59754, CAX11959, CAX11958, CAX11957, CAX14028, CAX14027, CAX14026, CAH73823, CAH73875, AAV41895 and BAF83443, all of which sequences are herein incorporated by reference in their entirety. For purposes of practicing the instant invention, Chk2 may comprise or consist of an amino acid sequence that is at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:17.

[0054] Chk1, which is encoded by the CHEK1 gene in humans, has an amino acid sequence as shown in Figure 4 (SEQ ID NO:18). Human Chk1 is also known in the art as "CHK1 checkpoint homolog (*S. pombe*)." SEQ ID NO:18 is presented for reference purposes only, since human Chk1 and other mammalian Chk1 protein sequences are known. For example, the NCBI website (or GenBank) discloses human Chk1 amino acid sequences under accession numbers AAC51736, AAW02681, AAH04202, BAF85238, BAA84577, CAB70558, 014757, AAM58752, AAM78553, CAD10662, AAH17575, AAE84492, CAZ65679, AAB88852, ABM83833, ABM87141, AAE67465, AAE67917, AAX36253, BAI45672, NP_001107594 and NP_001265, all of which sequences are herein incorporated by reference in their entirety. For purposes of practicing the instant invention, Chk1 may comprise or consist of an amino acid sequence that is at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:18.

[0055] cdc25c (cell division cycle 25C), which is a tyrosine phosphatase, is encoded by the CDC25C gene in humans and has an amino acid sequence as shown in Figure 5 (SEQ ID NO:19). Human cdc25c is also known in the art as "M-phase inducer phosphatase 3" and "cell division cycle 25 homolog C (*S. pombe*)." SEQ ID NO:19 is presented for reference purposes only, since human cdc25c and other mammalian cdc25c protein sequences are known. For example, the NCBI website (or GenBank) discloses human cdc25c amino acid sequences under accession numbers AAR32098, P30307, NP_001781, AAX36531, EAW62145, EAW62149, AAX29802, AAX29802, ABP29523, EAW62148, BAG63273, AAA75741, AAE74714, AAH19089 and AAA35666, all of which sequences are herein incorporated by reference in their entirety. For purposes of practicing the instant invention, cdc25c may comprise or consist of an amino acid sequence that is at least about 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:19.

[0056] While one aspect of the instant invention is drawn to blocking the downregulatory activity of activated Chk2 against cdc25c, it is similarly drawn to blocking the activity of Chk2 against one or more of its other protein targets. Additional Chk2 protein targets are E2F-1, p53, cdc25a, BRCA-1, PML, Che-1, Hdmx, Trf2, FoxM1, pRB or mdm2, for example. Further, the invention is drawn to blocking the downregulatory activity of Chk1 against not only cdc25c, but also p53, cdc25a, cdc25b, Rad51, poly-A-binding protein, aurora-B, tousled-like kinase-1, wee-1 or BLM, for example.

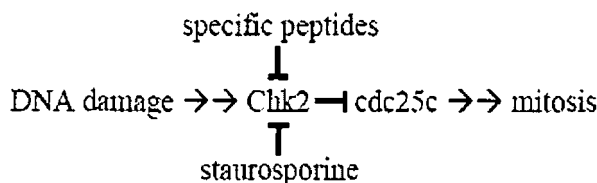
[0057] The following examples are included to demonstrate certain embodiments of the invention.

EXAMPLES

[0058] A study was conducted to identify short peptides applicable for preventing or reducing toxicity in the skin that

can result from exposure to a mutagen or irritant. Given the prior known inhibitory effects of caffeine on the DNA damage repair pathway and the anti-cancer effects of this inhibition in epidermis, certain related peptides were assayed for the ability to similarly inhibit the DNA damage repair pathway. To this end, peptides were tested for the ability to block the

Chk2 (checkpoint kinase 2) - cdc25c (cell division cycle 25 homolog C protein) arm of the DNA damage repair pathway. [0059] Chk2, which is activated by DNA damage-sensing proteins, induces cell cycle arrest by phosphorylating and thereby inhibiting cdc25c, which is a phosphatase involved in upregulating cyclin-dependent kinase activity leading to mitosis. The interaction of these proteins in the DNA damage repair pathway and their interaction with the agents discussed below is summarized in the following schematic:



[0060] Peptides were developed to inhibit Chk2 deactivation of cdc25c using the following methodology. All peptides were synthesized using standard Fmoc chemistry on an Advanced ChemTech (Louisville, KY) Apex 396 Multiple Peptide Synthesizer. After cleavage, the peptides were purified via HPLC using a reverse phase C-18 column and then analyzed by mass spectrometry. Primary sequence confirmation and preparative purification were accomplished using an LC/MS/MS system (ABI API2000). All of the peptides used in this particular example (SEQ ID NOs:2-6 and 8-10) were carboxy-terminal amidated (-CONH₂).

[0061] The inhibitory activity of SEQ ID NOs:2-6 and 8-10 on Chk2 phosphorylation of cdc25c was measured using the K-LISA™ Checkpoint Activity Kit available from Calbiochem/EMD Biosciences (San Diego, CA) per the manufacturer's instructions. Activated human Chk2 enzyme for these tests was obtained from Sigma (St. Louis, MO). The general kinase inhibitor, staurosporine (10 μM final concentration) was used as a positive control for the inhibition of Chk2 activity. Individual test peptides (SEQ ID NOs:2-6, 8-10) were incubated with Chk2 at a final concentration of 50 μg/mL. Briefly, the K-LISA™ assay utilized a biotinylated peptide substrate (KKKVSRSGLYRSPMPENLNRPR, SEQ ID NO:12) that can be phosphorylated on the third serine by Chk2 (can also be phosphorylated by Chk1 protein). This substrate served as a surrogate for cdc25c, since SEQ ID NO:12 contains the sequence targeted for serine phosphorylation by Chk2.

[0062] The biotinylated substrate peptide and sample containing Chk2 with or without added agents (staurosporine or one of SEQ ID NOs:2-6, 8-10) were incubated in the presence of ATP in wells of a streptavidin-coated 96-well plate. This incubation allowed for substrate phosphorylation by Chk2 and substrate capture in a single step. Following incubation, the phosphorylated substrate was detected using an anti-phosphoserine primary antibody, a horseradish peroxidase (HRP)-conjugated secondary antibody and TMB (tetramethylbenzidine) substrate for color development (the primary and secondary antibodies, and TMB, were provided in the K-LISA™ kit). Assay sensitivity was increased by the addition of the Stop solution provided in the kit. Relative Chk2 activity was determined by reading the absorbance of each well at dual wavelengths 450/540 nm.

[0063] SEQ ID NOs:2, 3, 5, 6, 8 and 10 all inhibited the activity of Chk2 against the substrate peptide (Figure 2). For example, SEQ ID NO:10 reduced Chk2 phosphorylation activity by about 48%, which approached the inhibitory activity exhibited by staurosporine (about 69%). SEQ ID NOs:5, 6 and 8 had a similar capacity to inhibit Chk2. Overall, these data indicate that certain peptides can reduce Chk2 activity towards its substrate (i.e., cdc25c), further indicating a capacity to reduce the level of cell cycle arrest that would be initiated *in vivo* upon cellular exposure to DNA-damaging agents.

[0064] All of the compositions or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of certain embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the methods described herein.

SEQUENCE LISTING

[0065]

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Zhang, Lijuan

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<150> US Provisional 61/262,790

<151> 2009-11-19

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		465				470											

Claims

1. A peptide composed of the sequence of SEQ ID NOs: 3, 5, 6, 8 or 10.
- 5 2. The peptide according to claim 1, wherein said peptide is SEQ ID NO: 10.
3. The peptide according to claims 1 or 2, wherein said peptide is a free acid or amidated at its carboxy terminus.
- 10 4. A composition comprising a peptide according to any one of claims 1 to 3 and a pharmaceutically acceptable carrier.
5. The composition of claim 4, wherein the composition is in the form of an aerosol, emulsion, liquid, lotion, cream, paste, ointment, powder, or foam.
- 15 6. The composition according to claims 4 or 5 for use in skin care treatment.
7. The composition according to claims 4 or 5 for use in maintaining healthy skin traits, such as tone, elasticity, hydration, coloration, firmness and smoothness.
- 20 8. The composition according to claims 4 or 5 for use in treating the effects of skin photoaging, such as wrinkles, mottling and/or hyperpigmentation, rough or leathery skin, drooping or sagging skin, yellowing dryness, and skin neoplasms.
- 25 9. A composition according to claims 4 or 5 or a peptide selected from the group consisting of: SEQ ID NO: 2, 3, 5, 6, 8 or 10 for use as a medicament.
- 30 10. A peptide for use in reducing or preventing mutagen-induced skin tissue toxicity associated with the activity of an activated cell cycle checkpoint kinase, wherein said peptide sequence is SEQ ID NO: 2, 3, 5, 6, 8 or 10.
- 35 11. The peptide for use according to claim 10, wherein said skin tissue toxicity is in the form of skin cancer, pre-cancerous lesions, erythema, alopecia, photosensitivity, recall reactions, blistering, skin necrosis, acneiform, xerosis, increased pigmentation, dermatitis, or skin ulcers..
12. A peptide for use in treating the skin of a mammal to reduce the toxicity associated with ultraviolet light exposure, wherein said peptide sequence is SEQ ID NO: 2, 3, 5, 6, 8 or 10.
- 40 13. A peptide for use in treating skin damaged by UV light exposure or maintaining healthy skin, wherein said peptide sequence is SEQ ID NO: 2, 3, 5, 6, 8 or 10.
14. The non-therapeutic use of a peptide selected from the group consisting of: SEQ ID NO: 2, 3, 5, 6, 8 or 10 as a cosmetic product.
15. A cosmetic product comprising a peptide selected from the group consisting of: SEQ ID NO: 2, 3, 5, 6, 8 or 10.

Patentansprüche

1. Peptid, das aus der Sequenz von SEQ ID NO: 3, 5, 6, 8 oder 10 zusammengesetzt ist.
2. Peptid nach Anspruch 1, wobei es sich bei dem Peptid um SEQ ID NO: 10 handelt.
3. Peptid nach Anspruch 1 oder 2, wobei das Peptid eine freie Säure oder an seinem Carboxy-Terminus amidiert ist.
4. Zusammensetzung, die ein Peptid nach einem der Ansprüche 1 bis 3 und einen pharmazeutisch unbedenklichen Träger umfasst.
5. Zusammensetzung nach Anspruch 4, wobei die Zusammensetzung in Form eines Aerosols, einer Emulsion, einer Flüssigkeit, einer Lotion, einer Creme, einer Paste, einer Salbe, eines Pulvers oder eines Schaums vorliegt.

6. Zusammensetzung nach Anspruch 4 oder 5 zur Verwendung bei der Hautpflegebehandlung.
7. Zusammensetzung nach Anspruch 4 oder 5 zur Verwendung bei der Aufrechterhaltung gesunder Hauteigenschaften wie Tonus, Elastizität, Hydrierung, Färbung, Festigkeit und Glattheit.
8. Zusammensetzung nach Anspruch 4 oder 5 zur Verwendung bei der Behandlung der Wirkungen von Lichtalterung der Haut wie Falten, Flecken und/oder Überpigmentierung, raue oder lederne Haut, welke oder schlaffe Haut, Vergilben, Trockenheit und Hauttumoren.
9. Zusammensetzung nach Anspruch 4 oder 5 oder Peptid, das aus der Gruppe ausgewählt ist, die aus SEQ ID NO: 2, 3, 5, 6, 8 oder 10 besteht, zur Verwendung als Medikament.
10. Peptid zur Verwendung bei der Verminderung oder Prävention von mutagen-induzierter Hautgewebetoxizität, die mit der Aktivität einer aktivierten Zellzyklus-Kontrollpunkt-Kinase assoziiert ist, wobei es sich bei der Peptidsequenz um SEQ ID NO: 2, 3, 5, 6, 8 oder 10 handelt.
11. Peptid zur Verwendung nach Anspruch 10, wobei die Hautgewebetoxizität in Form von Hautkrebs, präkanzerösen Läsionen, Erythemen, Alopezie, Lichtempfindlichkeit, Recall-Reaktionen, Blasenbildung, Hautnekrose, Akneiform, Xerose, erhöhter Pigmentierung, Dermatitis oder Hautgeschwüren vorliegen.
12. Peptid zur Verwendung bei der Behandlung der Haut eines Säugers, um die mit Bestrahlung durch ultraviolettes Licht assoziierte Toxizität zu verringern, wobei es sich bei der Peptidsequenz um SEQ ID NO: 2, 3, 5, 6, 8 oder 10 handelt.
13. Peptid zur Behandlung von Haut, die durch Bestrahlung mit UV-Licht geschädigt wurde, oder zum Aufrechterhalten einer gesunden Haut, wobei es sich bei der Peptidsequenz um SEQ ID NO: 2, 3, 5, 6, 8 oder 10 handelt.
14. Nicht-therapeutische Verwendung eines Peptids, das aus der Gruppe ausgewählt ist, die aus SEQ ID NO: 2, 3, 5, 6, 8 oder 10 besteht, als Kosmetikprodukt.
15. Kosmetikprodukt, das ein Peptid umfasst, das aus der Gruppe ausgewählt ist, die aus SEQ ID NO: 2, 3, 5, 6, 8 oder 10 besteht.

Revendications

1. Peptide composé de la séquence des SEQ ID n^{os}: 3, 5, 6, 8 ou 10.
2. Peptide selon la revendication 1, ledit peptide étant SEQ ID n° : 10.
3. Peptide selon les revendications 1 ou 2, ledit peptide étant un acide libre ou amidé à son extrémité carboxy.
4. Composition comprenant un peptide selon l'une quelconque des revendications 1 à 3 et un vecteur pharmaceutiquement acceptable.
5. Composition selon la revendication 4, la composition étant sous la forme d'un aérosol, d'une émulsion, d'un liquide, d'une lotion, d'une crème, d'une pâte, d'un onguent, d'une poudre ou d'une mousse.
6. Composition selon les revendications 4 ou 5 destinée à être utilisée en traitement de soins de la peau.
7. Composition selon les revendications 4 ou 5 destinée à être utilisée en maintien de traits de peau saine, tels que la tonicité, l'élasticité, l'hydratation, la coloration, la fermeté et le caractère lisse.
8. Composition selon les revendications 4 ou 5 destinée à être utilisée en traitement des effets du photovieillissement de la peau, tels que les rides, les mouchetures et/ou l'hyperpigmentation, la peau rugueuse ou tannée, la peau tombante ou s'affaissant, le jaunissement, la sécheresse et les néoplasmes de la peau.
9. Composition selon les revendications 4 ou 5 ou peptide choisi dans le groupe constitué par : SEQ ID n° : 2, 3, 5, 6,

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8 ou 10 destinés à être utilisés comme médicament.

5 **10.** Peptide destiné à être utilisé en réduction ou prévention de la toxicité induite par mutagenèse sur des tissus cutanés associée à l'activité d'une kinase de point de contrôle du cycle cellulaire activée, ladite séquence du peptide étant SEQ ID n° : 2, 3, 5, 6, 8 ou 10.

10 **11.** Peptide destiné à être utilisé selon la revendication 10, ladite toxicité sur des tissus cutanés étant sous la forme d'un cancer de la peau, de lésions précancéreuses, d'un érythème, d'alopécie, d'une photosensibilité, de réactions de réactivation, d'une vésication, d'une nécrose de la peau, d'une réaction acnéiforme, d'une xérose, d'une pigmentation accrue, d'une dermatite ou d'ulcères cutanés.

12. Peptide destiné à être utilisé en traitement de la peau chez un mammifère pour réduire la toxicité associée à l'exposition à de la lumière ultraviolette, la séquence dudit peptide étant SEQ ID n° : 2, 3, 5, 6, 8 ou 10.

15 **13.** Peptide destiné à être utilisé en traitement de peau endommagée par exposition à de la lumière UV ou en maintien de peau saine, la séquence dudit peptide étant SEQ ID n° : 2, 3, 5, 6, 8 ou 10.

20 **14.** Utilisation non thérapeutique d'un peptide choisi dans le groupe constitué par : SEQ ID n° : 2, 3, 5, 6, 8 ou 10 comme produit cosmétique.

15. Produit cosmétique comprenant un peptide choisi dans le groupe constitué par : SEQ ID n° : 2, 3, 5, 6, 8 ou 10.

SEQ ID NO:11

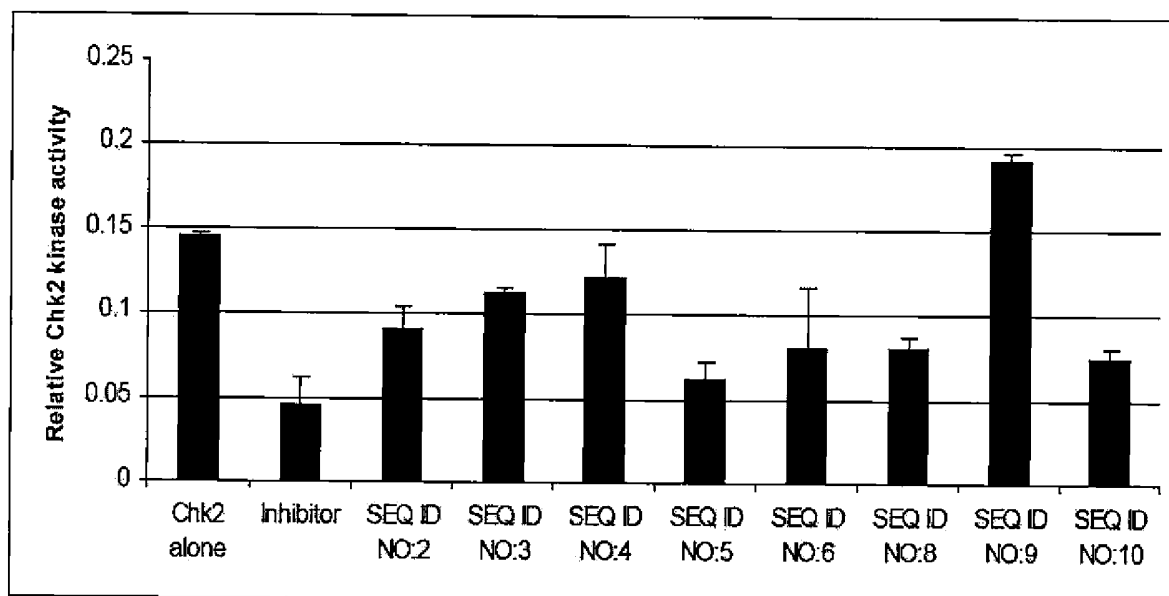
```

1  mllavlycll wsfqtsaghf pracvssknl mekeccppws gdrspcgqls grgscqmill
61  snaplgpqfp ftgvddresw psvfynrtcq csgnfmgnfc gnckfgfwgp ncterrllvr
121 rnifdlsape kdkffayltl akhtissdyv ipigtygqmk ngstpmfndi niydlfvwmh
181 yyvsmdallg gyeiwrldif aheapaflpw hrlfllrweq eiqlktgden ftipywdwrđ
241 aekcdictde ymggqhptnp nllspasffs swqivcsrle eynshqslcn gtpegplrrn
301 pgnhdkstrtp rlpssadvef clsltqyesg smdkaanfsf rntlegfasp ltgiadasqs
361 smhnalhiym ngtnsqvqgs andpifllhh afvdsifeqw lrrhrplqev ypeanapigh
421 nresymvpfi plyrngdffi sskdlgydys ylgdsdpdsf qdyiksyleq asriwswllg
481 aamvgavlta llaglvslc rhkrkqlpee kqpllmekedyhslyqshl

```

hslyqsh (SEQ ID NO:8)
 hslyqs (SEQ ID NO:9)
 slyqs (SEQ ID NO:10)
 fkslyqs (SEQ ID NO:7)
 fhslyqsh (SEQ ID NO:6)
 yhslyesk (SEQ ID NO:5)
 yhslyqshl (SEQ ID NO:2)
 dfhslyqsh (SEQ ID NO:4)
 yhslyqshi (SEQ ID NO:3)
 dyhtlyqthl (SEQ ID NO:1)

FIG. 1



Inhibitor: Staurosporine
Substrate: KKKVSRSGLYRSPSPENLNRPR
(SEQ ID NO:12)

FIG. 2

Human Chk2 (SEQ ID NO:17)

```

1  msresdveaq qshgssacsq phgsvtqsqq sssqsggiss sststmpnss qsshsssgtl
61 ssletvstqe lysipedqep edgepeepth apwarlwalq dgfanlecvn dnywfgdrks
121 ceycfdepll krtckyrtyt kkhfrifrev gpknsyiayi edhsgngtfv ntelvgkgkr
181 rplnnnseia lslnrnkvfv ffdltvddqs vypkalrdey imsktlgsga cgevklafer
241 ktckkvaiki iskrkfaigs areadpalnv eteieilkk1 nhpciikikn ffdaedyyiv
301 lelmeggelf dkvvgnkr1k eatcklyfyq mllavqylhe ngiihrdlkp envllssqee
361 dclikitdfg hskilgets1 mrtlcgtpty lapevlvs1g tagynravdc wslgvilfic
421 lsgyppfseh rtqvslkdqi tsgkynfipe vwaevsekal dlvkkl1vvd pkarftteea
481 lrhplw1qded mkrkfqdl1s eenestalp1 v1aqpstsrk rpregeaega ettkrpavca
541 avl

```

FIG. 3

Human Chk1 (SEQ ID NO:18)

```

1  mavpfvedwd lvqtlgegay gevqlavnrv teeavavkiv dmkravdcpe nikkeicink
61 mlnhenvvkf yghrregniq ylfleycsgg elfdrieppi gmpepdagr fhlmagvvy
121 lhgigithrd ikpenlllde rdnlkisdfg latvfrynnr erllnkmcgt lpyvapellk
181 rrefhaepvd vwscgivlta mlagelpwdq psdscqeyds wkekktynp wkkidsapla
241 llhkilvenp saritipdik kdrwynkplk kgakrprvts ggvsespqsf skhiqsnldf
301 spvnsassee nvkysssqpe prtglslwdt spsyidklvq gisfsqptcp dhmllnsqll
361 gtpgssqnpw qrlvkrmtrf ftkldadksy qclketcekl gyqwkksmnn qvtisttdrr
421 nnklifkvn1 lemddkilvd frlskgdgle fkrhflkikg klidivssqk vwlpat

```

FIG. 4

Human cdc25c (SEQ ID NO:19)

```

1  mstelfsstr eegssgsgps frsnqrkmln lllerdtsft vcpdvprtpv gkflgdsanl
61  silsggtpkr cldlsnlssg eitatqltts addidetghld ssglqevhla gmnhdqhlmk
121 cspaqllest pngldrghrk rdamcsssan kendngnlvd semkylgspi ttvpkldknp
181 nlgedqaeai sdelmefslk dgeakvsrsg lyrspsmphen lnrprlkqve kfkdnrtipdk
241 vkkkyfsggg klrkgclclkk tvslcditit qmleedsnqg hligdfskvc alptvsgkhq
301 dlkyvnpetv aallsgkfqq liekfyvidc rypyeylggh iggalnlysq eelfnfflkk
361 pivpldtqkr iiivfhcefs sergprmcrc lreedrslng ypalyypely ilkggyrdff
421 peymelcepg sycpmhhqdh ktellrcrsq skvqegerql regiallvkd msp

```

FIG. 5

REFERENCES CITED IN THE DESCRIPTION

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