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(71) Applicant (for all designated States except US): **THE GOVERNMENT OF THE UNITED STATES OF AMERICA AS REPRESENTED BY THE SECRETARY OF THE DEPARTMENT OF HEALTH AND HUMAN SERVICES, CENTERS FOR DISEASE CONTROL AND PREVENTION** [US/US]; Technology Transfer Office, 4770 Buford Highway (k79), Atlanta, Georgia 30341 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **DING, Min** [US/US]; 1014 Brettwald Drive, Morgantown, West Virginia 26508 (US).

(74) Agent: **GORDON, Debra, A.; KLARQUIST SPARKMAN, LLP**, ONE WORLD TRADE CENTER, SUITE 1600, 121 Sw Salmon Street, Portland, Oregon 97204 (US).

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(54) Title: CYANIDIN-3-GLUCOSIDE AS AN ANTI-NEOPLASTIC AGENT

(57) Abstract: This disclosure concerns the discovery that cyanidin-3-glucoside inhibits neoplastic transformation, metastasis, neoplastic cell migration and invasion, activation of tumor cell markers {e.g., NF-κB, AP-I, COX-2, TNF-α and MAPK}, activation of cell migration markers (e.g., JNK, p38, and ERK), and induces apoptosis in neoplastic cell (such as HL-60 cells). Cyanidin-3-glucoside is also demonstrated to possess strong antioxidant activity involving, at least, inhibition reactive oxygen species and induction of cytoprotective genes.



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CYANIDIN-3-GLUCOSIDE AS AN ANTI-NEOPLASTIC AGENT**CROSS REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of U.S. Provisional Application No.
5 60/643,371, filed January 11, 2005, which application is incorporated herein in its entirety.

ACKNOWLEDGMENT OF GOVERNMENT SUPPORT

This invention was made by the Department of Health and Human Services,
10 Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Health Effects Laboratory Division, an agency of the United States Government.

FIELD OF THE DISCLOSURE

15 This disclosure relates to methods of using cyanidin-3-glucoside to inhibit neoplasm and/or metastasis, and related compositions.

BACKGROUND

Plants and plant extracts have been used for centuries for medicinal
20 purposes, and bio-prospecting continues in modern times. Identification and characterization of an individual therapeutic property and the responsible compound is a resource-intensive and unpredictable system, but it is vital in the on-going search for therapeutic agents to ameliorate diseases and other detrimental health conditions.

25 Cancer is a major cause of death in both men and women. Significant efforts have been, and continue to be, made to find new approaches for treating this disease family. Many naturally occurring substances present in the human diet have been identified as potential chemopreventive agents (Ross and Kasum, *Annu. Rev. Nutr.*, 22:19, 2002; Saleem *et al.*, *Nutr. Cancer*, 47:13, 2003; Gupta and Mukhtar, *Skin. Pharmacol. Appl. Skin Physiol.*, 14:373, 2001; Kelloff *et al.*, *J. Nutr.*, 130:4678,
30 2000). Epidemiological studies indicate that consumption of phytochemicals (compounds derived from plants, such as fruits and vegetables) might reduce the

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incidence of cancers and other chronic diseases (Cal *et al.*, *Curr. Med. Chem. Anticancer Agents*, 3:77, 2003; Morse and Stoner, *Carcinogenesis*, 14:1737, 1993; Cline and Hughes, *Cancer Treat. Res.*, 94:107, 1998).

Anthocyanins are a group of naturally occurring phenolic compounds
5 belonging to the flavonoid class of molecules (Ross and Kasum, *Annu. Rev. Nutr.*, 22:19, 2002). Anthocyanins are pigments present in many berries, in dark grapes, cabbages and other pigmented foods. Epidemiological studies show that a moderate consumption of complex mixtures of anthocyanins, such as red wine or bilberry extract, is related to the low risk of cardiovascular disease (Morazzoni and
10 Bormbardelli, *Fitoterapia*, 67:3, 1996; Renud and de Logeril, *Lancet*, 339:1523, 1992; Muth *et al.*, *Altern. Med. Rev.*, 5:164, 2000; Hou, *Curr. Mol. Med.*, 3:149, 2003). Mixtures of anthocyanins (such as in plant extract) have also been shown to have anti-neoplastic activity (*e.g.*, Kang *et al.*, *Cancer Lett.*, 194:13-19, 2003; Nair *et al.*, U.S. No. 6,656,914, issued December 2, 2003; Shirai *et al.*, U.S. Pat. App.
15 Pub. No. 2004/0053859, published March 18, 2004; Leahy *et al.*, U.S. Pat. App. Pub. No. 2002/0054924, published May 9, 2002; Afaq *et al.*, *Int. J. Cancer*, 113:423-433, 2005) and anti-oxidant activity (*e.g.*, Sashwati *et al.*, *Free Radical Res.*, 36:1023-1031, 2002; Taruscio *et al.*, *J. Agric. Food Chem.*, 52:3169-3176, 2004).

20 Less is known about the preventative and/or therapeutic effects of particular anthocyanidins, such as cyanidin-3-glucoside (C3G). Like other anthocyanins, C3G is naturally occurring in many foods, such as fruits and berries. For example, C3G is found in blueberries and represents about 80% of the total anthocyanins present in blackberries (Dugo, *J. Agric. Food Chem.*, 49:3987, 2001).

25 New cancer therapeutics and preventive agents are needed. Compounds present in edible foodstuffs offer a safe and fertile source of such agents.

SUMMARY OF THE DISCLOSURE

30 Cyanidin-3-glucoside is an ingredient of various health-promoting fruits and vegetables, such as blackberry. This disclosure concerns the discovery that cyanidin-3-glucoside inhibits neoplastic transformation, metastasis, cell migration and invasion, and activation of tumor markers NF- κ B, COX-2, TNF- α , AP-1 and

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MAPK, and induces apoptosis in neoplastic cells. Cyanidin-3-glucoside also possesses strong antioxidant activity and can inhibit generation of reactive oxygen species and induce an antioxidant protective response.

In view of its newly discovered properties, cyanidin-3-glucoside can be used to treat neoplasia in subjects (such as, humans). The applicability of cyanidin-3-glucoside to the *in vivo* treatment of neoplasia, as described in particular embodiments herein, is clear because aspects of the discovery have been demonstrated in living subjects. Exemplar neoplasias that can be treated using cyanidin-3-glucoside include skin cancer and lung cancer.

In the course of determining the anti-neoplastic effects of cyanidin-3-glucoside, the inventors also surprisingly discovered that cyanidin-3-glucoside not only treats or inhibits neoplasia, but also reduces or avoids metastasis of malignant cells. Thus, in other applications of the discovery, cyanidin-3-glucoside is used to inhibit metastasis of neoplasms (such as, lung cancer and skin cancer).

Furthermore, because cyanidin-3-glucoside is known to be present in edible foodstuffs, this compound is expected to be a well-tolerated and safe therapy for treatment of neoplasia (such as skin or lung cancer) and metastasis. This benefit of cyanidin-3-glucoside is in stark contrast to traditional chemotherapy methods, many of which have toxic side effects.

The foregoing and other features and advantages will become more apparent from the following detailed description of several embodiments, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a series of bar graphs (A-C) demonstrating a dose-dependent effect of C3G on three different indicators of viability in HL-60 human promyelocytic leukemia cells (grey bars) and non-neoplastic macrophage cells (black bars). FIG. 1A shows the percentage of HL-60 cells and macrophage cells undergoing apoptosis after treatment with C3G from 0 to 80 μ M. FIG. 1B shows MTT uptake by HL-60 cells after treatment with C3G from 0 to 15 μ M. FIG. 1C shows caspase-

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3 activity (as measured by Ac-DEVD-AMC fluorogenic substrate) in HL-60 cells and macrophage cells after treatment with C3G from 0 to 80 μ M.

FIGs. 2A-G are a series of bar graphs demonstrating effects of C3G on UVB- or TPA-induced AP-1, NF- κ B, COX-2, TNF- α , and MAPKs activation.

5 Mouse epidermal JB6 cell line was stably transfected with an AP-1, NF- κ B, COX-2, or TNF- α luciferase reporter plasmid. The stable transfectants were pretreated with C3G for 1 hour followed by exposure to TPA (20 nm) or UVB radiation (4 kJ/m²) for 24 hours. The AP-1 (A and B), NF- κ B (C and D), COX-2 (E), or TNF- α (F) activities were measured by luciferase assay. Results, presented as relative AP-1, 10 NF- κ B, COX-2, or TNF- α activity compared to untreated control cells, are expressed as means and standard errors from three wells. FIG. 2G shows the effect of C3G on MAPKs activation. Cells were treated as described above and MAPKs activation were analyzed by Western blot with a phospho-specific antibody specific for phosphorylated sites.

15 **FIG. 3** shows two graphs demonstrating the effect of C3G on TPA-induced transformation and tumorigenesis in DMBA/TPA-treated mice. In particular, FIG. 3A shows the effect of C3G on TPA-induced neoplastic transformation in JB6 cells by soft agar analysis. JB6 P⁺ cells were exposed to TPA (20 ng/ml) with or without the indicated concentrations of C3G on soft agar medium for 14 days. The 20 cell colonies were scored by a computerized image analyzer. FIG. 3B is a time course showing the number of papillomas observed in control (closed circle), TPA-treated (open circle), and TPA- and C3G-treated (closed triangles) mice over a period of 21 weeks.

FIG. 4A-E demonstrate the effect of C3G on *in vitro* and *in vivo* A549 25 tumor cell growth (A and B, respectively), wound healing (C), cell migration (D) and cell invasion (E).

FIG. 5A is a Western blot showing a dose-dependent effect of C3G on Nrf2 protein expression. **FIG. 5B** is a bar graph showing GST activity as a function of C3G concentration.

30 **FIG. 6A** demonstrates the effect of C3G on UVB-induced activation of MAPKs in a human lung cancer cell, A549. **FIG. 6B** demonstrates the effect of C3G on TPA-induced ERK activation in A549 cells.

DETAILED DESCRIPTION

I. Introduction

Disclosed herein are methods for preventing or treating a neoplasm of an exposed body surface (such as the skin or the cervix) in a subject (such as a mammal or, in particular examples, a human). Such methods involve administering a therapeutically effective amount of cyanidin-3-glucoside to the subject, thereby inhibiting the development or growth of or otherwise treating the neoplasm in the subject. In certain embodiments, a therapeutically effective amount of cyanidin-3-glucoside is an amount sufficient to avoid the development or inhibit the progression of a target tumor. For example, such an amount would provide target tissue concentrations that have been found effective *in vitro*. In particular embodiments, a therapeutically effective amount of topically applied cyanidin-3-glucoside is from about 0.2% to about 1% cyanidin-3-glucoside (w/w).

In some method embodiments, the neoplasm is cervical cancer (such as a cervical papilloma induced by human papilloma virus), papilloma, basal cell carcinoma, squamous cell carcinoma, melanoma, cutaneous T-cell lymphoma (mycosis fungoides), apocrine carcinoma of the skin, or Merkel cell carcinoma. In more particular examples, the neoplasm is chemically induced (such as, a TPA-induced neoplasm). In other method embodiments, treating the neoplasm involves inhibiting metastasis of the neoplasm and/or reducing the size of the neoplasm.

Certain method embodiments involve treating (including inhibiting the development of) a neoplasm of an exposed body surface in a subject by topically applying cyanidin-3-glucoside to the exposed body surface prior to exposure of the exposed body surface to an agent that promotes development of the neoplasm. In specific examples, an agent that promotes development of the neoplasm is ultraviolet radiation and cyanidin-3-glucoside is applied prior to ultraviolet radiation exposure.

Also disclosed are methods of inhibiting metastasis of a malignant cell by exposing at least one malignant cell to an amount of cyanidin-3-glucoside sufficient to inhibit metastasis (such as from about 8 to about 10 mg/kg cyanidin-3-glucoside).

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In some examples, the malignant cell originates from a lung neoplasm or a skin neoplasm. In other embodiments, exposing at least one malignant cell to an amount of cyanidin-3-glucoside sufficient to inhibit metastasis involves administering cyanidin-3-glucoside to a subject. In particular embodiments, administering
5 cyanidin-3-glucoside to a subject includes intrathecal, intradermal, intramuscular, intraperitoneal (ip), intravenous (iv), subcutaneous, intranasal, epidural, or enteral (such as, oral) administration, or combinations thereof.

Also contemplated herein are topical compositions including cyanidin-3-glucoside and an agent capable of blocking ultraviolet radiation (such as
10 para-aminobenzoate (PABA), ethylhexyl methoxycinnamate, DEA methoxycinnamate, padimate O, ethylhexyl salicylate, homosalate, TEA salicylate, oxybenzone, dioxybenzone, sulisobenzene, avobenzone, octocrylene, titanium dioxide, zinc oxide or menthyl anthranilate, or combinations thereof). Some topical
15 compositions may also contain one or more emollients or antioxidants, or a combination thereof.

II. Abbreviations and Terms

AMC	7-amino-4-methyl coumarin
C3G	cyanidin-3-glucoside
C3R	cyanidin-3-glucosylrutinoside
HPV	human papilloma virus
MTT	3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide
PMN	polymorphonuclear
ROS	reactive oxygen species
TPA	12- <i>O</i> -tetradecanolyphorbol-13-acetate
UV	ultraviolet

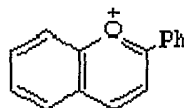
Unless otherwise noted, technical terms are used according to conventional
20 usage. To facilitate review of the various embodiments of the invention, the following explanations of specific terms are provided:

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Agent: Any substance (such as, an atom, molecule, molecular complex, chemical, peptide, protein, protein complex, nucleic acid, or drug) or any combination of substances that is useful for achieving an end or result, such as a therapeutic end or result.

- 5 **Agent capable of blocking ultraviolet radiation:** An agent that protects a body surface exposed to UV radiation (such as, the skin) from at least some of the harmful effects of ultraviolet radiation exposure. Some such agents may commonly be called “sunscreens.” UV-blocking agents contain molecules that absorb the harmful wavelengths of ultraviolet light before they can reach the exposed body
- 10 surface (e.g., the skin). Although not bound by theory, it is commonly understood that the absorbed light is converted to heat and rapidly dissipated to the skin and environment, which allows these molecules to revert to a lower energy state, and subsequently absorb another photon of light.

- Anthocyanidins:** Oxygenated derivatives of flavylum
- 15 (2-phenylchromenylium) salts, having the general chemical structure:



- Anthocyanidins are aglycons of anthocyanins. An “**aglycon**” is the non-sugar compound remaining after replacement of the glycosyl group from a glycoside by a hydrogen atom. Exemplar anthocyanidins include cyanidin, delphinidin, petunidin,
- 20 pelargonidin,
 peonidin and malvidin.

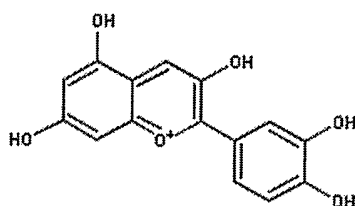
- Anthocyanins:** Plant pigments of the flavonoid class, which are glycosides of anthocyanidins. A “**glycoside**” is any compound that contains a carbohydrate molecule (such as, a monosaccharide or lower oligosaccharide), and can be
- 25 hydrolytically cleaved into its carbohydrate and a non-carbohydrate (aglycone) components. Glycosides are typically named for the carbohydrate contained in the molecule, for example, glucoside (for glucose-containing glycosides), pentoside (for pentose-containing glycosides), or fructoside (for fructose-containing glycosides).

- Anti-neoplastic:** Having anti-tumor activity, for example inhibiting the
- 30 development or progression of a tumor, including local tumor growth or recurrence or metastatic spread.

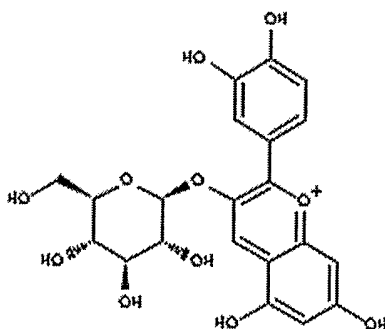
- 8 -

Cancer or Neoplasia: A biological condition in which a neoplasm has undergone characteristic anaplasia with loss of differentiation, increased rate of growth, invasion of surrounding tissue, and which is capable of metastasis. A “**neoplasm**” is an abnormal growth of cells or tissue, particularly a new growth of cells or tissue in which the growth is uncontrolled and progressive. A tumor is an example of a neoplasm. A “**chemically induced neoplasm**” is an abnormal growth of cells initiated and/or promoted by a chemical carcinogen. Specific exemplar carcinogens (including chemical carcinogens) are described elsewhere in this specification. Specific neoplasms are also described throughout this specification.

Cyanidin: An anthocyanidin having the following chemical structure:



Cyanidin can be glycosylated, for example, at the hydroxyl group at position 3, to form an anthocyanin. Cyanidin-3-glucoside (CAS Registry No. 7084-24-4) is formed by the transfer of a glucosyl group to the 3-OH group of cyanidin, and has the following structure:



Glycoside: Originally mixed acetal resulting from the attachment of a glycosyl group to a non-acyl group RO- (which itself may be derived from a saccharide). A “**glucoside**” is a glycoside in which the sugar constituent is glucose.

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Glycosyl group: The structure obtained by removing the hydroxy group from the hemiacetal function of a monosaccharide (such as, glucose) or lower oligosaccharide. A “**glucosyl**” group is the structure obtained by removing the hemiacetal (C-1) hydroxy group from glucose (also called, a glucose radical).

5 **Inhibit or inhibiting:** With respect to disease (such as neoplasm or metastasis), either term includes (i) preventing the disease, *e.g.*, causing the clinical symptoms of the disease not to develop in a subject that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease, (ii) restraining the disease, *e.g.*, arresting the development of the disease or
10 its clinical symptoms, or (iii) relieving the disease, *e.g.*, causing regression of the disease or its clinical symptoms.

Malignant cell: A neoplastic cell characterized by the ability to invade surrounding tissues and spread (or metastasize) from a site of a primary neoplasm.

Metastasis: The process by which malignant cells transfer from one organ
15 or part of the body to a separate organ or part of the body. This term also refers to a growth of malignant cells distant from the site of the primary neoplasm from which the malignant cells arose.

Subject: Living multicellular, vertebrate organism, a category which includes both human and veterinary subjects for example, mammals, rodents, and
20 birds.

Therapeutically effective amount: A quantity of a specified agent sufficient to achieve a desired effect in a subject being treated with that agent. For example, this may be the amount of cyanidin-3-glucoside necessary to inhibit a neoplasm or metastasis of a malignant cell. Ideally, a therapeutically effective
25 amount of an agent is an amount sufficient to effect the desired result without causing a substantial cytotoxic effect in the subject. The effective amount of an agent useful for preventing or treating a neoplasm or inhibiting metastasis of a malignant cell will be dependent on the subject being treated, the severity of the affliction, and the manner of administration of the therapeutic composition (as
30 described in more detail elsewhere in this specification).

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Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the subject matter of this disclosure belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise.

5 "Comprising" means "including." Hence "comprising A or B" means "including A or B," or "including A and B." All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety to the full extent permitted by applicable law. In case of conflict, the present specification, including explanations of terms, will control.

10 Except as otherwise noted, the methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. Materials, methods, and examples are illustrative only and not intended to be limiting.

15

III. Cyanidin-3-Glucoside

Cyanidin-3-glucoside is shown herein to possess anti-neoplastic activity in both *in vivo* and *in vitro* cancer models, and to inhibit metastasis of malignant cells. These and other properties of cyanidin-3-glucoside can be exploited in methods for
20 treating or inhibiting the development of neoplasms and inhibiting metastases therefrom. In addition, cyanidin-3-glucoside can be included in skin-protective topical compositions, which can be applied to an exposed body surface, for example, prior, during or after exposure of a subject to a carcinogen (such as TPA or ultraviolet radiation) or even after the development of neoplasm (or metastasis
25 thereof). In this manner, a topical composition containing cyanidin-3-glucoside is useful to treat, prevent or restrain a neoplasm (or metastasis thereof).

Cyanidin-3-glucoside for use in the disclosed methods or compositions is commercially available from several sources including, for instance, CarboMer, Inc. (San Diego, CA, USA), and Apin Chemicals (Abingdon, Oxon, UK). In addition,
30 cyanidin-3-glucoside is an anthocyanin found in many plants, particularly in the fruits and berries thereof. Accordingly, cyanidin-3-glucoside can be isolated from any such plant or its fruit or berry using methods commonly known in the art.

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Representative fruits and berries that contain cyanidin-3-glucoside include blueberries, strawberries, bilberries, cranberries, grapes, raspberries, elderberries, cherries, apples, purple corn, pomegranates, currants, gooseberries, chokeberries, rowanberries, blackberries, beans (such as black beans), grapefruit, eggplant, dogwood fruits (aka, Cornelian cherries), oranges (for instance, *Moro*, *Sanguinello* or *Tarocco* oranges). Particular plant families, the fruit or berries of which are thought to contain cyanidin-3-glucoside, include without limitation Grossulariaceae, Ericaceae, Rosaceae, Empetraceae, Elaeagnaceae, and Caprifoliaceae. Particular plant genera containing cyanidin-3-glucoside include, for example, *Rubus* (such as, *Rubus fruticosus*), *Ribes* (such as, *R. nigrum*, *Ribes x pallidum* Otto and F. Dier (cv. Red Dutch or cv. White Dutch), and *Ribes uva-crispa*), *Vaccinium* (such as *Vaccinium uliginosum*, *Vaccinium myrtillus*, *Vaccinium corymbosum*, *Vaccinium vitis-idaea*, and *Vaccinium oxycoccos*), *Aronia* (such as *Aronia mitschurinii*), *Prunus* (such as *Prunus spinosa*), *Empetrum* (such as *Empetrum hermaphroditum*, and *Empetrum nigrum*), *Hippophae* (such as *Hippophae rhamnoides*), and *Sambucus* (such as *Sambucus nigra*).

One representative method for isolating cyanidin-3-glucoside from a plant (or its fruit or berry) involves high performance liquid chromatography, as described in more detail in the Examples. Other methods for isolating cyanidin-3-glucoside from fruits or berries are commonly known (see, e.g., Escribano-Bailon *et al.*, *Phytochem. Anal.*, 13:354-357, 2002).

IV. Methods of Using Cyanidin-3-Glucoside

As this disclosure demonstrates, cyanidin-3-glucoside inhibits neoplastic transformation, metastasis, cell migration and invasion, activation of the tumor markers (e.g., NF- κ B, AP-1, COX-2, TNF- α , and MAPK), activation of cell migration markers (e.g., JNK, p38, and ERK), and induces apoptosis in neoplastic cells. Cyanidin-3-glucoside also possesses strong antioxidant activity and can inhibit generation of reactive oxygen species and induce an antioxidant protective response. Accordingly, cyanidin-3-glucoside is useful to treat or inhibit the development of neoplasms (such as, skin or lung cancer), and/or inhibit metastasis of malignant cells. It has now been found that cyanidin-3-glucoside is particularly

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effective as an anti-neoplastic agent on body surfaces. In some methods, a therapeutically effective amount of cyanidin-3-glucoside is administered to a subject to inhibit the development of or treat an existing neoplasm of an exposed body surface. Additional methods involve contacting one or more malignant cell(s) with
5 cyanidin-3-glucoside to inhibit metastasis of the malignant cell(s).

Any living, multicellular, vertebrate organism capable of developing one or more neoplasm(s) is contemplated as a subject for the disclosed methods. Thus, in particular examples, a subject of a disclosed method is a human or veterinary subject. Veterinary subjects include, without limitation, mammals (such as, rodents,
10 canines, felines, bovines, ovines, or equines, or combinations thereof), fishes, or birds.

Cyanidin-3-glucoside can be used to treat or prevent, or inhibit metastasis from, any neoplasm. Non-limiting examples of neoplasms include tumors of the skin (such as, squamous cell carcinoma, basal cell carcinoma, melanoma, skin
15 appendage tumors, papilloma, cutaneous T-cell lymphoma (mycosis fungoides), apocrine carcinoma of the skin, or Merkel cell carcinoma), breast carcinomas (*e.g.* lobular and duct carcinomas), and other solid tumors, sarcomas, and carcinomas of the lung like small cell carcinoma, large cell carcinoma, squamous carcinoma, and adenocarcinoma, mesothelioma of the lung, colorectal adenocarcinoma, stomach
20 carcinoma, prostatic adenocarcinoma, ovarian carcinoma such as serous cystadenocarcinoma and mucinous cystadenocarcinoma, ovarian germ cell tumors, testicular carcinomas, and germ cell tumors, pancreatic adenocarcinoma, biliary adenocarcinoma, hepatocellular carcinoma, bladder carcinoma including transitional cell carcinoma, adenocarcinoma, and squamous carcinoma, renal cell
25 adenocarcinoma, endometrial carcinomas including adenocarcinomas and mixed Mullerian tumors (carcinosarcomas), carcinomas of the endocervix, ectocervix, and vagina such as adenocarcinoma and squamous carcinoma, esophageal carcinoma, carcinomas of the nasopharynx and oropharynx including squamous carcinoma and adenocarcinomas, salivary gland carcinomas, brain and central nervous system
30 tumors including tumors of glial, neuronal, and meningeal origin, tumors of peripheral nerve, soft tissue sarcomas and sarcomas of bone and cartilage, and non-solid hematopoietic tumors, such as leukemias.

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Particular method embodiments involve the use of cyanidin-3-glucoside to treat a neoplasm of an exposed body surface. As used herein, an "exposed body surface" is any part of the body capable of direct exposure to neoplastic promoters present in the environment (such as, UV radiation, neoplasm-inducing viruses (*e.g.*,
5 HPV), or chemical carcinogens). Neoplasms treatable by the disclosed methods may arise on any exposed body surface including, for example, the skin, the cervix, the vagina, mouth, nose, ears, or combinations thereof. Non-limiting examples of neoplasms of an exposed body surface include papilloma, basal cell carcinoma, squamous cell carcinoma, melanoma, cutaneous T-cell lymphoma (mycosis
10 fungoides), apocrine carcinoma of the skin, or Merkel cell carcinoma.

Particular method embodiments contemplate the treatment or prevention of skin neoplasm(s) by administering cyanidin-3-glucoside (exemplar methods of cyanidin-3-glucoside administration are discussed below). Skin cancer is the most common type of cancer in the United States and, in many cases, is associated with
15 and promoted by exposure of the skin to ultraviolet radiation. Some traditional treatments for skin cancer involve topical chemotherapy with anticancer drugs in a lotion or cream applied to the skin. One such skin cancer treatment involves fluorouracil applied to the skin daily for several weeks. Intense inflammation is common as a result of fluorouracil treatment. Advantageously, cyanidin-3-glucoside
20 occurs naturally in fruit and berries and is well tolerated when contacted to the skin (or other exposed body surface) of a subject.

A neoplasm, as contemplated in the disclosed methods, can arise by any biological mechanism. Many agents capable of promoting neoplasm (for example, of an exposed body surface) are commonly known. Such agents can include, for
25 instance, biological agents (such as viruses, like human papilloma virus), radiation (such as UVA, UVB, or γ -radiation), or chemical carcinogens. Carcinogenic agents include, without limitation, 12-*O*-tetradecanolyphorbol-13-acetate (TPA), acetaldehyde, 2-acetylaminofluorene, acrylamide, acrylonitrile, doxorubicin hydrochloride, aflatoxins, 2-aminoanthraquinone, o-aminoazotoluene,
30 4-aminobiphenyl, 1-amino-2-methylanthraquinone, 2-amino-3-methylimidazo[4,5-f]quinoline, amitrole, o-anisidine hydrochloride, arsenic compounds, inorganic, asbestos, azacitidine, azathioprine, benzene, benzdine and

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dyes metabolized to benzidine, benzotrichloride, beryllium and beryllium compounds, bromodichloromethane, 2,2-bis(bromoethyl)-1,3-propanediol, 1,3-butadiene, 1,4-butanediol dimethylsulfonate, butylated hydroxyanisole (BHA), cadmium and cadmium compounds, carbon tetrachloride, chlorambucil, 5 chloramphenicol, chlorendic acid, chlorinated paraffins (C12, 60% chlorine), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU), bis(chloroethyl) nitrosourea, chloroform, bis(chloromethyl) ether and technical-grade chloromethyl methyl ether, 3-chloro-2-methylpropene, 4-chloro-o-phenylenediamine, chloroprene, p-chloro-o-toluidine and 10 p-chloro-o-toluidine hydrochloride, chlorozotocin, chromium hexavalent compounds, cisplatin, p-cresidine, cupferron, cyclophosphamide, cyclosporin A, dacarbazine, danthron (1,8-dihydroxyanthraquinone), 2,4-diaminoanisole sulfate, 2,4-diaminotoluene, 1,2-dibromo-3-chloropropane, 1,2-dibromoethane (ethylene dibromide), 2,3-dibromo-1-propanol, tris(2,3-dibromopropyl) phosphate, 1,4- 15 dichlorobenzene, 3,3'-dichlorobenzidine and 3,3'-dichlorobenzidine dihydrochloride, dichlorodiphenyltrichloroethane; (DDT), 1,2-dichloroethane (ethylene dichloride), dichloromethane (methylene chloride), 1,3-dichloropropene, diepoxybutane, diethyl sulfate, diethylstilbestrol, diglycidyl resorcinol ether, 3,3'-dimethoxybenzidine and dyes metabolized to 3,3'-dimethoxybenzidine, 20 4-dimethylaminoazobenzene, 3,3'-dimethylbenzidine and dyes metabolized to 3,3'-dimethylbenzidine, dimethylcarbamoyl chloride, 1,1-dimethylhydrazine, dimethyl sulfate, dimethylvinyl chloride, 1,4-dioxane, disperse blue 1, epichlorohydrin, erionite, ethylene oxide, ethylene thiourea, di(2-ethylhexyl) phthalate, ethyl methanesulfonate, furan, glycidol, hexachlorobenzene, 25 hexachloroethane, hexamethylphosphoramide, hydrazine and hydrazine sulfate, hydrazobenzene, isoprene, chlordecone, lead acetate and lead phosphate, lindane and other hexachlorocyclohexane isomers, melphalan, methoxsalen with ultraviolet A therapy (PUVA), 2-methylaziridine (propylenimine), 4,4'-methylenebis(2-chloroaniline), 4,4'-methylenebis(N,N-dimethyl)benzenamine, 4,4'- 30 methylenedianiline and its dihydrochloride salt, methyleugenol, methyl methanesulfonate, N-methyl-N'-nitro-N-nitrosoguanidine, metronidazole, 4,4'-(dimethylamino)benzophenone, mirex, mustard gas, 2-naphthylamine, nickel

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- compounds and metallic nickel, nitrilotriacetic acid, o-nitroanisole, nitroarenes (*e.g.*, 1,6-dinitropyrene, 1,8-dinitropyrene, 6-nitrochrysene, 1-nitropyrene, 4-nitropyrene), nitrofen (2,4-dichlorophenyl-p-nitrophenyl ether), nitrogen mustard hydrochloride, 2-nitropropane, N-nitrosodi-n-butylamine, N-nitrosodiethanolamine,
- 5 N-nitrosodiethylamine, N-nitrosodimethylamine, N-nitrosodi-n-propylamine, N-nitroso-N-ethylurea, 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone, N-nitroso-N-methylurea, N-nitrosomethylvinylamine, N-nitrosomorpholine, N-nitrosornicotine, N-nitrosopiperidine, N-nitrosopyrrolidine, N-nitrososarcosine, norethisterone, ochratoxin A, 4,4'-oxydianiline, oxymetholone, phenacetin,
- 10 phenazopyridine hydrochloride, phenolphthalein, phenoxybenzamine hydrochloride, phenytoin, polybrominated biphenyls (PBBs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (*e.g.*, benz[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]acridine, dibenz[a,j]acridine, dibenz[a,h]anthracene, 7H-dibenzo[c,g]carbazole,
- 15 dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene, 5-methylchrysene), procarbazine hydrochloride, 1,3-propane sultone, β -propiolactone, propylene oxide, propylthiouracil, reserpine, safrole, selenium sulfide, streptozotocin, styrene-7,8-oxide, sulfallate, tamoxifen, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), tetrachloroethylene
- 20 (perchloroethylene), tetrafluoroethylene, tetranitromethane, thioacetamide, thiotepa, thiourea, thorium dioxide, toluene diisocyanate, o-toluidine and o-toluidine hydrochloride, toxaphene, trichloroethylene, 2,4,6-trichlorophenol, 1,2,3-trichloropropane, ultraviolet radiation (*e.g.*, UVA or UVB), uranium (insoluble or soluble compounds), vinyl bromide, vinyl chloride, vinyl cyclohexene dioxide,
- 25 vinylidene chloride (1,1-dichloroethylene), zinc chromate and hexavalent chromium compounds.

- Exemplar methods involve treating, preventing, or inhibiting metastasis of an environmentally induced neoplasm, such as a chemically induced neoplasm (including, for instance, a neoplasm induced, in whole or in part, by TPA exposure),
- 30 or a radiation-induced neoplasm (including, for instance, a neoplasm resulting, in whole or in part, from exposure to ultraviolet (*e.g.*, UVA or UVB) radiation, or a

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neoplasm induced by a pathogen, such as a virus (for example, human papilloma virus).

Treatment of a neoplasm using a disclosed method can involve, for example, inhibiting the growth of the neoplasm, reducing the size of the neoplasm, inducing

5 apoptosis of the neoplasm, or inhibiting metastasis of the neoplasm. Inhibiting the growth of a neoplasm conveys a wide-range of inhibitory effects that an agent (*e.g.*, cyandin-3-glucoside) may have on the initiation and growth of a neoplasm, for example, as compared to an untreated (or pre-treatment) neoplasm. Thus, inhibiting the growth of a neoplasm includes situations wherein an incidence of neoplasm is

10 reduced or the normal growth rate of the neoplasm has slowed (for example, the number of neoplastic cells still increases over time, but not as rapidly as in a control neoplastic cell population (*e.g.*, pretreatment)), equals zero (for example, there is substantially no change in number of neoplastic cells in the population over time; for instance, neoplastic cell growth is approximately equal to cell death or quiescence in

15 the same population), or becomes negative (for example, the number of neoplastic cells decreases over time; for instance, cell death exceeds cell growth or quiescence). A reduction in the size of a neoplasm can be determined using any methods or standard known to the ordinarily skilled artisan. In one embodiment, the decrease in one or more physical dimensions of a neoplasm (such as, diameter,

20 volume, length, width, or weight) as compared to corresponding measurement(s) made at an earlier time point (such as pre-treatment or earlier in a course of treatment) can indicate a neoplasm size reduction. Inhibiting metastasis of a neoplasm (or malignant cells thereof) conveys a wide-range of inhibitory effects that an agent (*e.g.*, cyandin-3-glucoside) may have on metastasis of such neoplasm (or

25 malignant cells). For example, inhibiting metastasis may be considered relative to an untreated (*i.e.*, uninhibited or control) rate of metastasis of a particular malignant cell or population of malignant cells of interest. Thus, inhibiting metastasis includes situations wherein the metastatic rate of a cell or cell population has slowed (*i.e.*, the number metastatic cells decreases over time as compared to a control population), or

30 is reduced to near zero (*i.e.*, there are substantially no metastatic cells in the population over time).

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A. Administration of Cyanidin-3-Glucoside

This disclosure contemplates administering to a subject cyanidin-3-glucoside as an anti-neoplastic agent, for example, to treat or inhibit the development of (for example, prevent) a neoplasm. Any cyanidin-3-glucoside delivery system or treatment regimen that effectively treats or inhibits the development of a neoplasm (or metastasis) of interest can be used. Similarly, cyanidin-3-glucoside-containing compositions can be formulated in any manner known in the art. Exemplar cyanidin-3-glucoside formulations may include diluent(s), excipient(s) or carrier(s), or one or more additional ingredients, such as UV-blocking agents, antioxidants, emollients, or fragrances. The specific dose level and frequency of dosage for any particular subject may be varied and will depend upon a variety of factors, including the activity of the specific compound, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, and severity of the condition of the subject receiving therapy. An attending healthcare worker (or the subject) may elect to modify the concentration and/or dosage in order to adjust the dose to the particular response of each subject. Exemplar modes of administration, formulations and dosage regimens applicable to the disclosed methods and compositions are discussed in more detail below.

1. Topical Administration and Topical Formulations

Particular method embodiments envision topical application of a therapeutic agent containing cyanidin-3-glucoside. The terms "topical application," "topically applied" and the like are used interchangeably herein to refer to the application of an agent onto an exposed body surface (such as, an outer layer of mammalian skin or the cervix). The compositions can be applied onto a body surface using any known or otherwise effective application technique including, but not limited to, the techniques of rubbing, brushing, painting, wiping, and stroking a composition onto the skin.

When cyanidin-3-glucoside is administered in a cutaneous or topical carrier or diluent, the carrier or diluent may be chosen from any known in the cosmetic or medical arts; for example, any gel cream, lotion, ointment, liquid or non liquid carrier, emulsifier, solvent, liquid diluent or other similar vehicle which does not

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exert deleterious effect on the skin or other living animal tissue. The carrier or diluent is usually a mixture of several ingredients, including, but not limited to liquid alcohols, liquid glycols, liquid polyalkylene glycols, water, liquid amides, liquid esters, liquid lanolin, lanolin derivatives and similar materials. Alcohols
5 include mono and polyhydric alcohols, including ethanol, glycerol, sorbitol, isopropanol, diethylene glycol, propylene glycol, ethylene glycol, hexylene glycol, mannitol and methoxyethanol. Typical carriers may also include ethers (such as, diethyl and dipropyl ether), methoxypolyoxyethylenes, carbowaxes, polyethyleneglycerols, polyoxyethylenes and sorbitols. In some embodiments, the
10 topical carrier includes both water and alcohol in order to maximize the hydrophylic and lipophylic solubility (for instance, a mixture of ethanol or isopropanol with water). One skilled in the art may choose other carriers or diluents to adapt to specific dermatologic needs.

A topical carrier may also include various other ingredients commonly used
15 in ointments and lotions and well known in the cosmetic or medical arts; for example, agents capable of blocking ultraviolet radiation (*e.g.*, sunscreens), antioxidants, fragrances, perfumes, gelling agents, thickening agents (such as carboxymethylcellulose), surfactants, stabilizers, emollients, coloring agents and other similar agents.

20 In some examples a topical composition contains one or more agents capable of blocking UV radiation (such as, UVA or UVB radiation, or both). Such agents include, without limitation, para-aminobenzoate (PABA) and its derivatives, ethylhexyl methoxycinnamate, DEA methoxycinnamate, padimate O, ethylhexyl salicylate, homosalate, TEA salicylate, oxybenzone, dioxybenzone, sulisobenzene,
25 avobenzene, octocrylene, titanium dioxide, zinc oxide or menthyl anthranilate. In other embodiments, a topical composition contains at least one UVA-blocking agent, such as oxybenzone, dioxybenzone, sulisobenzene, avobenzene or zinc oxide. In still other embodiments, a topical composition includes at least one
UVB-blocking agent, such as substituted para-aminobenzoates (*e.g.*, octyl dimethyl
30 PABA), alkyl esters of para-methoxycinnamate (*e.g.*, octyl para-methoxycinnamate), certain esters of salicylic acid (*e.g.*, homomenthyl salicylate or octyl salicylate), ethylhexyl methoxycinnamate, DEA methoxycinnamate, padimate

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O, ethylhexyl salicylate, homosalate, TEA salicylate, octocrylene or titanium dioxide.

In particular embodiments, a topical composition contains one or more antioxidants such as, Vitamins A and E, or their esters, magnesium ascorbyl
5 phosphate, DL panthenol, beta glucan, propyl, octyl or dodecyl esters of gallic acid, butylated hydroxyanisole (usually as a mixture of ortho and meta isomers), butylated hydroxytoluene or nordihydroguaiaretic acid.

In other embodiments, a topical composition includes one or more emollients. Non-limiting representative emollients include mineral oil, lanolin oil,
10 coconut oil, cocoa butter, olive oil, almond oil, macadamia nut oil, aloe extract, jojoba oil, safflower oil, corn oil, liquid lanolin, cottonseed oil, peanut oil, purcellin oil, perhydrosqualene, castor oil, polybutene, odorless mineral spirits, sweet almond oil, calophyllum oil, ricin oil, vitamin E acetate, mineral spirits, the oil of cereal germs (such as the oil of wheat germ), and esters such as isopropyl palmitate,
15 isopropyl myristate, butyl myristate, hexadecyl stearate, decyl oleate, acetyl glycerides, the octanoates and benzoates of (C12-C15) alcohols, the octanoates and decanoates of alcohols and polyalcohols such as those of glycol and glycerol, ricin oleates of alcohols and poly alcohols, such as those of isopropyl adipate, hexyl laurate and octyl dodecanoate.

20 2. Other Useful Methods of Administration and Formulations

Other methods of administering a therapeutic agent disclosed herein (such as cyanidin-3-glucoside) include parental or enteral routes, such as intrathecal, intradermal, intramuscular, intraperitoneal (ip), intravenous (iv), subcutaneous, intranasal, epidural, and oral routes. The therapeutics may be administered by any
25 convenient route, including, for example, infusion or bolus injection, absorption through epithelial or mucocutaneous linings (for example, oral mucosa, rectal and intestinal mucosa, and the like), ophthalmic, nasal, and transdermal, and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce a pharmaceutical
30 composition by any suitable route, including intraventricular and intrathecal injection. Intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir. Pulmonary administration can also be

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employed (for example, by an inhaler or nebulizer), for instance using a formulation containing an aerosolizing agent.

In a specific embodiment, it may be desirable to administer a therapeutic agent locally to the area in need of treatment. This may be achieved by, for
5 example, local or regional infusion or perfusion during surgery, topical application (as discussed in additional detail above), injection, catheter, suppository, or implant (for example, implants formed from porous, non-porous, or gelatinous materials, including membranes, such as sialastic membranes or fibers), and the like. In one embodiment, administration can be by direct injection at the site (or former site) of a
10 tissue that is to be treated, such as the cervix. In another embodiment, the therapeutic are delivered in a vesicle, such as liposomes (see, e.g., Langer, *Science*, 249:1527, 1990; Treat *et al.*, in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez Berestein and Fidler (eds.), Liss, N.Y., pp. 353 365, 1989).

In yet another embodiment, the therapeutic can be delivered in a controlled
15 release system. In one embodiment, a pump may be used (see, e.g., Langer, *Science*, 249:1527, 1990; Sefton, *Crit. Rev. Biomed. Eng.*, 14:201, 1987; Buchwald *et al.*, *Surgery*, 88:507, 1980; Saudek *et al.*, *N. Engl. J. Med.*, 321:574, 1989). In another embodiment, polymeric materials can be used (see, e.g., Ranger *et al.*, *Macromol. Sci. Rev. Macromol. Chem.*, 23:61, 1983; Levy *et al.*, *Science*, 228:190, 1985;
20 During *et al.*, *Ann. Neurol.*, 25:351, 1989; Howard *et al.*, *J. Neurosurg.*, 71:105, 1989). Other controlled release systems, such as those discussed in the review by Langer (*Science*, 249:1527, 1990), can also be used.

The vehicle in which the agent is delivered can include pharmaceutically acceptable compositions known to those with skill in the art. For instance, in some
25 embodiments, therapeutic agents disclosed herein are contained in a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" means approved by a regulatory agency of the federal or a state government or listed in the U.S. Pharmacopoeia or other generally recognized pharmacopoeia for use in animals, and, more particularly, in humans. The term "carrier" refers to a diluent,
30 adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil,

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mineral oil, sesame oil, and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions, blood plasma medium, aqueous dextrose, and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. The medium may also contain
5 conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, lipid carriers such as cyclodextrins, proteins such as serum albumin, hydrophilic agents such as methyl cellulose, detergents, buffers, preservatives and the like.

Examples of pharmaceutical excipients include starch, glucose, lactose,
10 sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, and the like. The therapeutic, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The therapeutic can take the form of solutions, suspensions, emulsion, tablets, pills, capsules,
15 powders, sustained release formulations, and the like. The therapeutic can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. A more complete explanation of
20 parenteral pharmaceutical carriers can be found in Remington: The Science and Practice of Pharmacy (19th Edition, 1995) in chapter 95.

The ingredients in various embodiments are supplied either separately or mixed together in unit dosage form, for example, in solid, semi-solid and liquid dosage forms such as tablets, pills, powders, liquid solutions, or suspensions, or as a
25 dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water or saline can
30 be provided so that the ingredients may be mixed prior to administration.

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3. Therapeutically Effective Amounts and Dosage Regimens

Therapeutic preparations will contain a therapeutically effective amount of at least one active ingredient (such as, cyanidin-3-glucoside) together with a suitable amount of carrier so as to provide proper administration to the patient. The
5 formulation should suit the mode of administration.

The amount of the therapeutic that will be effective depends on the nature of the disorder or condition to be treated, as well as the stage of the disorder or condition. Effective amounts can be determined by standard clinical techniques. The precise dose to be employed in the formulation will also depend on the route of
10 administration, and should be decided according to the judgment of the health care practitioner and each patient's circumstances. For example, the concentration of active ingredient (*e.g.*, cyanidin-3-glucoside) in a topical composition (such as, an ointment, cream, gel or lotion) is typically from about 0.2% to about 1% (by weight relative to the total weight of the topical composition, *e.g.*, ointment, cream, gel or
15 lotion); for example, from about 0.3% to about 0.9%, from about 0.4% to about 0.8%, and from about 0.5% to about 0.7%. Within the preferred ranges, higher concentrations allow a suitable dosage to be achieved while applying the lotion, ointment, gel or cream in a lesser amount or with less frequency. In other embodiments, a dosage range for non-topical administration (such as, oral
20 administration, or intravenous or intraperitoneal injection) of a composition containing cyanadin-3-glucoside is from about 0.1 to about 200 mg/kg body weight in single or divided doses; for example from about 1 to about 100 mg/kg, from about 2 to about 50 mg/kg, from about 3 to about 25 mg/kg, or from about 5 to about 10 mg/kg. In still other embodiments, cyanidin-3-glucoside is administered in
25 such a manner and/or amount as to achieve a target tissue cyanidin-3-glucoside concentration from about 1 μ M to about 50 μ M; for example, from about 2 μ M to about 40 μ M, from about 3 μ M to about 30 μ M, or from about 5 μ M to about 20 μ M.

The therapeutic agents of the present disclosure can be administered at about
30 the same dose throughout a treatment period, in an escalating dose regimen, or in a loading-dose regime (for example, in which the loading dose is about two to five times the maintenance dose). In some embodiments, the dose is varied during the

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course of a treatment based on the condition of the subject being treated, the severity of the disease or condition, the apparent response to the therapy, and/or other factors as judged by one of ordinary skill in the art. In some embodiments long-term treatment with a disclosed therapeutic composition is contemplated, for instance in
5 order to prevent reoccurrence of a neoplasm (such as, a skin or cervical lesion).

In some embodiments, a disclosed therapeutic agent (including cyanidin-3-glucoside) is administered to a subject before, concurrent with and/or after exposure to an agent that promotes neoplasm. It may be useful to administer the therapeutic agent at least 30 minutes, at least 60 minutes, at least 90 minutes, at
10 least 2 hours, at least 6 hours, or at least 1 day prior to exposure to a carcinogenic agent. In other embodiments, the therapeutic agent is first (or again) administered at any time after exposure to an agent that promotes neoplasm; for example, the therapeutic agent may be administered up to about 1 hour, up to about 6 hours, up to about 12 hours, up to about 1 day, or even longer after exposure to the carcinogenic
15 agent.

In more particular embodiments involving topical use of a disclosed therapeutic agent (including cyanidin-3-glucoside), a lotion, ointment, gel or cream containing the agent is thoroughly rubbed into the skin and the skin is preferably not washed in that region for at least 30 minutes (such as, for at least 1 hour or for at
20 least 4 hours). In this and other examples, the topical composition may be applied to the affected region from 1 to 6 times daily, for instance, 3 times daily at approximately regular intervals.

The following examples are provided to illustrate certain particular features
25 and/or embodiments. These examples should not be construed to limit the invention to the particular features or embodiments described.

EXAMPLES

Example 1

30 ISOLATION OF C3G

This Example demonstrates an exemplar method for isolating C3G from blackberry (*Rubus fruticosus*).

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High performance liquid chromatography (HPLC) was used to separate and isolate C3G from blackberry fruit tissue. Fruit samples were extracted twice with 80% methanol, 0.1% HCl in a Polytron™ homogenizer (Brinkmann Instruments, Inc., Westbury, NY) for 1 minute. Extracts were combined and concentrated by using a Buchler Evapomix™ (Fort Lee, NJ) in a water bath at 35°C. The concentrated sample was dissolved in acidified water (0.1% HCl) and then passed through a C₁₈ Sep-Pak™ cartridge (Waters), which was previously activated with methanol followed by water and then 0.1% HCl. C3G and other anthocyanins were adsorbed onto the column while sugars, acids, and other water-soluble compounds were eluted with 0.1% HCl. The C3G and other anthocyanins were then recovered with acidified (0.1% HCl) methanol. The methanol extract was passed through a 0.45 µm membrane filter (Millipore, MSI, Westboro, MA) and then separated by HPLC.

A Waters (Waters Associated, Millipore, Milford, MA) HPLC system equipped with two pumps (600 E system Controller) coupled with a photodiode array detector (Waters 2996 Series) was used. Samples were injected at ambient temperature (20°C) onto a reverse phase NOVA-PAK™ C₁₈ column (150H3.9 mm, particle size 4 µm) with a guard column (NOVA-PAK™ C₁₈, 20x3.9 mm, particle size 4 µm) (Sentry guard holder universal). The mobile phase was water containing 10% formic acid and methanol (90/10, V/V). The flow rate was 1 mL/min.

The C3G fraction was identified by the characteristic C3G UV spectra, recorded with a diode-array-detector, and by chromatographic comparison with a C3G standard. The C3G-containing fraction was collected, lyophilized and stored at -70°C for later use. C3G isolated as described in this example was 99% pure.

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Example 2

C3G SPECIFICALLY INHIBITS NEOPLASTIC CELL GROWTH

This Example demonstrates that C3G specifically inhibited the growth of human promyelocytic leukemia HL-60 cell and induced apoptosis in these cells. In contrast, C3G did not promote apoptosis in normal rat lung alveolar macrophages or blood PMN cells. C3G may also stimulate differentiation of HL-60 cells.

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An MTT assay was used to assess the viability of cells treated with C3G. This colorimetric assay system measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells (for review, see *e.g.*, Hayon *et al.*, *Leuk. Lymphoma*, 44(11):1957-1962, 2003; Sargent, 5 *Recent Results Cancer Res.*, 161:13-25, 2003). After incubation of the cells with the MTT reagent in the absence or presence of 2.5 to 15 μ M C3G for approximately 48 hours, a detergent solution was added to lyse the cells and solubilize the colored crystals. The lysed samples were read using an ELISA plate reader at a wavelength of 590 nm. The amount of color produced is directly proportional to the number of 10 viable cells. As shown in FIG 1B, C3G inhibited the proliferation of HL-60 in a dose-dependent manner.

To assess apoptosis in C3G-treated cells, HL-60 cells and normal rat lung alveolar macrophages were incubated with 0, 20, 40 or 80 μ M C3G for 16 hours. Typical apoptotic nuclear morphology was determined by staining with 10 μ mol/L 15 bis-benzimide Hoechst 33258 fluorochrome for 30 minutes. The percentages of apoptotic cells were determined under the fluorescence microscope. As shown in FIG. 1A, HL-60 cells, but not normal macrophages, undergo apoptosis in the presence of C3G.

Caspase 3 is a member of a family of cysteine aspartic acid-specific 20 proteases, which have been shown to play a key role in apoptosis of mammalian cells. Fluorogenic substrates for caspase 3, such as an 7-amino-4-methyl coumarin (AMC)-labeled substrate can be detected in a sample by exposure to UV light at 360 nm. AMC is released from these substrates upon cleavage by caspase 3 enzymes. Free AMC produces a yellow-green fluorescence that is monitored by a 25 fluorometer at 460 nm. The amount of yellow-green fluorescence produced upon cleavage is proportional to the amount of caspase 3 activity present in the sample.

An AMC fluorescence assay was used to determine the amount of caspase 3 activity in HL-60 and normal rat lung alveolar macrophages in the absence and presence of C3G. In particular, cells were treated with C3G for 16 hours at 37°C, 30 collected by centrifugation, washed, and lysed for 30 minutes on ice. Cell lysates were centrifuged and the supernatants were used for caspase activity assay. The caspases 3-like activities were determined by incubating 230 μ l of assay buffer with

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50 μ l of supernatant and 10 μ l of Ac-DEVD-AMC Fluorogenic Substrate (BD Biosciences, San Diego, CA). After incubation for 1 hour at 37°C in 96-well plates, the fluorescence of the free AMC released upon proteolytic cleavage of the substrate by the appropriate caspase was detected at 380 nm excitation and 460 nm emissions, using a Cary Eclipse Fluorescence Spectrophotometer equipped with microplate reader accessory (Varian). Caspase 3-like activity was expressed as the relative fluorescence (Arbitrary units).

As shown in FIG. 1C, C3G selectively induced caspase 3 activation in HL-60 cells, but not in the normal rat lung alveolar macrophages. Although not bound by theory, these results indicate that C3G-induced HL-60 cell apoptosis may be mediated by caspase 3 signaling.

Example 3

C3G INHIBITS UVB- OR TPA-INDUCED ACTIVATION OF AP-1, NF- κ B, COX-2, TNF- α , AND MAPK FAMILY MEMBERS

It is known that AP-1, NF- κ B, COX-2 and TNF- α play critical roles in tumorigenesis induced by tumor promoters (Li *et al.*, *Cancer Res.*, 57:3569, 1997; Jang *et al.*, *Science*, 275:218-220, 1997; Young *et al.*, *Proc. Natl. Acad. Sci. USA*, 96:9872, 1999). An antineoplastic agent may inhibit the expression of such biological markers. This Example demonstrates that C3G inhibits activation of biological markers of tumorigenesis, including AP-1, NF- κ B, COX-2 and TNF- α and MAPK family members and the upstream regulator, MKK4.

The JB6 mouse epidermal cell line, JB6, was stably transfected with AP-1, NF- κ B, COX-2, or TNF- α luciferase reporter plasmid. The stable transfectants were then pretreated with C3G for 1 hour followed by exposure to TPA (20 nm) or UVB radiation (4 kJ/m²) for 24 hours. The AP-1, NF- κ B, COX-2, or TNF- α activity was measured by luciferase assay. For the effect of C3G on MAPKs activation, cells were treated as described above and MAPKs activation were analyzed by Western blot with phospho-specific antibody against phosphorylated sites (New England Biolabs, Beverly, MA). Phosphorylated and non-phosphorylated proteins were detected using the same transferred membrane blot following a stripping procedure.

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As shown in FIGs. 2A-D, C3G caused a dose-dependent inhibition of AP-1 and NF- κ B activities induced by UVB or TPA. Similarly, UVB-induced COX-2 and TNF- α activities were inhibited by C3G in a dose-dependent manner (see FIGs. 2E and 2F). FIG. 2G shows that C3G inhibited TPA-induced phosphorylation of ERKs and UVB-induced phosphorylation of all members of MAPK family, including ERKs, p38 and JNKs. FIG. 2H shows that C3G also inhibited UVB-induced phosphorylation of MKK4, an upstream regulator of the MAPK family.

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Example 4

C3G INHIBITS TUMORIGENESIS IN DMBA/TPA-TREATED MICE

This Example demonstrates that C3G inhibits TPA induced cell transformation in a soft-agar *in vitro* model and in an *in vivo* model of carcinogenesis.

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JB6 P⁺ cells (1×10^4) were exposed to TPA (20 ng/ml) in the absence or presence of C3G (from 5 to 80 μ M) on soft agar medium for 14 days. The cell colonies were scored by a computerized image analyzer. As shown in FIG. 3A, C3G inhibited the number of TPA-treated cells capable of growth in soft agar in a dose-dependent manner.

20

The anti-tumorigenesis activity of C3G *in vivo* was demonstrated using the two-stage mouse skin cancer model in which DMBA and TPA were used as initiator and promoter, respectively (Balmain and Pragnell, *Nature*, 303:72, 1983; Naito *et al.*, *Carcinogenesis*, 4:639, 1988; Ding *et al.*, *J. Biol. Chem.*, 279:10670, 2004). Male and female mice of 6-9 weeks old were used. Dorsal skin of the mice was shaved and 2 days later a single dose of 400 nmol 7,12-dimethylbenz[a]anthracene (DMBA) dissolved in 300 μ l of acetone was topically applied. Fourteen days following initiation, the mice (except the negative control group) were promoted by dermal exposure to 17 nmol of TPA in 350 μ l of acetone twice a week for 22 weeks. For the C3G-treated group, the dorsal skin was pretreated topically with C3G (3.5 μ mol/mouse dissolved in 350 μ l acetone) 30 minutes before each application of TPA. The negative control group was treated with acetone only. The incidence of papillomas was detected by palpation and the number of papillomas appearing on

30

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each mouse was recorded once a week. At the end of the experiment, all the animals were sacrificed by intraperitoneal injection of pentobarbital (6.5 mg/mouse). For histopathology, the largest tumors were removed and fixed in freshly prepared 4% paraformaldehyde followed by paraffin embedding and subjected to pathological studies.

FIG. 3B shows that animals treated with C3G had fewer tumors per mouse. Significant differences were observed on and after 16 weeks following TPA promotion. In addition to the differences in the numbers of tumors, the size of tumor was significantly smaller in the C3G treated group. At the end of the experiment, there were five tumors greater than 4-5 mm in diameter in non-treated group; whereas, no large tumors were found in the C3G-treated group. Pathology study indicated that the tumors were squamous cell carcinoma.

Example 5

C3G INHIBITS A549 *IN VITRO* CELL GROWTH AND TUMOR XENOGRAFT GROWTH IN ATHYMIC NUDE MICE

This Example demonstrates that C3G inhibits the growth of A549 human lung cancer cells both *in vitro* and *in vivo*.

ECIS (Model 1600R, Applied BioPhysics, Troy, NY) assay, a widely used technique for measurement of cell attachment, spreading, and proliferation (Lo *et al.*, *Biophys. J.*, 69:2800-2807, 1995; Smith *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 91:5094-5098, 1994), was employed to monitor the growth of cultured cells. A549 cells (1×10^4) suspended in 400 μ l of medium without or with (2, 10 or 40 μ M) C3G were seeded on electrodes. The electrodes were pre-coated with the same medium for 0.5 hour before use. The cells were equilibrated in the incubator for 15 minutes. A constant current source applied an AC signal of 1 μ A at 4 kHz between a small active electrode (250 μ m diameter) and a large counter electrode to complete the circuit. The rate of cell proliferation on the microelectrode was monitored for 72 hours as real-time changes in resistance.

As shown in FIG. 4A, proliferation of A549 cells was significantly suppressed by C3G in a dose-dependent manner. At the concentration of 40 μ M

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C3G, cell growth was completely inhibited to the negative control level. Thus, C3G dramatically inhibits proliferation of A549 human lung cancer cells *in vitro*.

To further demonstrate the anti-neoplastic activity of C3G *in vivo*, C3G was used to treat A549 tumor xenografts in athymic male nude mice. Male nude mice (AthymicBCR-nu) aged 8 weeks were used, in 10 groups. All animals were housed in autoclaved plastic filter-top cages and were provided with autoclaved tap water and Prolab 3500 feed *ad lib*. Human lung cancer cell line, A549 cells, were subcutaneously injected in both right and left flanks of each mouse (2×10^6 cells/flank) to initiate tumor growth. After two days, the mice were treated intraperitoneally with either PBS or C3G dissolved in PBS (9.5 mg/kg, 3 times/week). Once tumor xenografts started growing, their sizes were measured twice weekly in two dimensions throughout the study. The tumor volume was calculated by formula: $0.5236 L_1(L_2)^2$, where L_1 is the long diameter and L_2 is the short diameter. Tumor volume (mm^3) was represented as mean of 10 mice in each group. As shown in FIG. 4B, C3G inhibited size of A549 tumor xenograft growth in athymic nude mice.

Metastatic dissemination into the other organs was evaluated macroscopically and by microscopic examination of tissue sections. Pathology indicated that tumors invaded through the abdominal wall and extended into the abdominal cavity, resulting in peritoneal carcinomatosis. Multiple small tumor nodules were observed on the peritoneal surface of the abdominal wall. The tumors also extensively involved the mesenteric fat with malignant ascites. Not only on the surface but also the parenchyma of several organs, such as liver, kidney, pancreas, peri-gastric lymph nodes were involved by the tumor. In contrast to control mice, C3G-treated mice showed much less tumor involvement of the abdominal cavity and a smaller subcutaneous tumor mass at the injection site was observed. The tumor nodules of C3G-treated mice were much less in the abdominal cavity or on the mesenteric fat. Microscopically, the injection site of the C3G treated mice show collection of macrophages with pigments, consistent with the phagocytosed pigment derived from C3G compound. There were viable tumor cells in the deep skeletal muscle distant from the subcutaneous injection site in C3G-treated group. There was no tumor involvement of organ parenchyma in the C3G-treated mice.

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Example 6

C3G INHIBITS METASTASIS OF MALIGNANT CELLS

Cell migration and invasion assays described in this Example further
5 demonstrate that C3G inhibits cell metastasis.

Wound healing assay: A549 cells were grown on cover slips to 100%
confluent monolayers and then scratched to form a 100 μm "wound" using sterile
pipette tips. The cells were then cultured with or without C3G in a serum free media
for 12 hours and fixed on coverslips with 4% formalin. Images were taken using an
10 Olympus photomicroscope.

Transwell migration assays: Cell-migration assays were conducted as
described by Qian *et al.* (*Am. J. Physiol. Cell Physiol.*, 286(1):C153-C163, 2004)
with slight modification. Briefly, the transwells were coated with ECL cell
attachment matrix (Upstate Biotechnology) at 20 $\mu\text{g/ml}$. The top chambers of the
15 transwells were loaded with 0.2 ml of cells (5.0×10^5 cells/ml) in 5% serum media
and the bottom chambers contained 0.6 ml of 10% serum media. The cells were
incubated in the transwells with or without C3G at 37°C in 5% CO_2 for 16 hours.
Migrating cells were fixed, stained with 0.1% crystal violet, and followed by dye
elution (10% acetic acid). A microplate reader was used to measure the O.D. of the
20 eluted solutions to determine the migration values. Mean values were obtained from
three individual experiments and were subjected to t-test.

C3G significantly blocked A549 cell migration in both wound healing assays
(FIG.4C) and transwell assays (FIGs. 4D and 4E). The potency of inhibition for cell
migration was 25% at 40 μM of C3G and 70% at 80 μM of C3G in transwell assays.
25 The cell invasion assays demonstrated that C3G was able to inhibit cell invasion in a
dose-dependent manner, 57% at 40 μM and 85% at 80 μM as measured by
Matrigel invasion assays. Together, the results shown in this Example and in
Example 5 establish that C3G inhibits cancer cell metastasis *in vivo* and *in vitro*.

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Example 7

C3G IS A POTENT ANTIOXIDANT

Various plant-derived compounds are believed to function as anti-cancer agents through antioxidant activities (Loo, *J. Nutr. Biochem.*, 14:64, 2003). Briefly, total antioxidant capacity of C3G was determined using an ABTS (2, 2'-aziodiethylbenzthiazoline sulfonate) test set (Randox Laboratories Ltd., UK). The principle of the assay depends on production of the radical cation ABTS⁺ in incubation medium containing the substrates (H₂O₂ and peroxidase), which is blue-green color and can be detected at 600 nm. Antioxidants in the sample cause the suppression of this color production to a degree that is proportional to their concentrations. The assays were calibrated against standards and expressed as micromoles per liter. C3G (0.5 mM) had a total antioxidant activity equivalent to 2.5 mM ascorbate.

H₂O₂ and O₂⁻ are two key reactive oxygen species (ROS) produced in response to UVB irradiation (Huang *et al.*, *J. Biol. Chem.*, 276:40234-40240, 2001). To further demonstrate the antioxidant properties of C3G, a cell culture system was used to investigate the effect of C3G on UVB-induced H₂O₂ and O₂⁻ production by confocal microscopy with specific fluorescence dyes (Ye *et al.*, *J. Biol. Chem.*, 274:26661, 1999; Wang *et al.*, *Am. J. Physiol.*, 279:C868, 2000). Briefly, JB6 cells were seeded onto a glass slip in the bottom of a well of a 24-well plate for 24 hours. The cells were pretreated with C3G for 30 minutes and then exposed to UVB in the present of dihydroethidium (2 μM) or DCFH-DA (5 μM). The cells were washed and fixed with 10% buffered formalin. The glass slip was mounted on a microscope slide and observed under a sarastro 2000 (Molecular Dynamics, Inc., Sunnyvale, CA) laser scanning confocal microscope fitted with an argon-ion laser. C3G inhibited the generation of both H₂O₂ and O₂⁻ in JB6 cells in a dose-dependent manner.

The transcription factor Nrf2 is believed to play an essential role in the antioxidant response element (ARE)-mediated expression of phase 2 detoxifying enzymes and stress-inducible genes (Itoh *et al.*, *Biochem. Biophys. Res. Commun.*, 236:313-322, 1997; Kobayashi *et al.*, *Methods. Enzymol.*, 378:273, 2004; Nguyen *et al.*, *Ann. Rev. Pharmacol. Toxicol.*, 43:233, 2003). Inducers of phase 2 and antioxidative enzymes are thought to enhance the detoxication of environmental

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carcinogens in animals; often leading to protection against neoplasia (Kensler, *Environ. Health Perspect.*, 105:965-970, 1997; Kwak *et al.*, *Mol. Cell. Biol.*, 22:2883-2892, 2002; Balogun *et al.*, *Biochem. J.*, 371:887-895, 2003).

The effect of C3G on Nrf2 nuclear translocation and Nrf2 transcriptional
5 activity was examined by Western Blot analysis or Nrf2 luciferase reporter system. Briefly, cells were treated with or without C3G and harvested. The cells were suspended in hypotonic buffer A (10 mM HEPES (pH 7.6), 10 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), 0.5 mM phenylmethylsulfonyl fluoride) for 10 minutes on ice. Nuclei were pelleted by centrifugation at 12,000 xg for
10 20 seconds and were resuspended in buffer C (20 mM HEPES (pH 7.6), 25% glycerol, 0.4 M NaCl, 1 mM EDTA, 1 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride) for 30 minutes on ice. The supernatants containing nuclear proteins were collected after centrifugation. Proteins that were extracted from either whole cell lysate (30 µg) or nuclei (30 µg) were separated by SDS-PAGE, transferred to
15 nitrocellulose membranes, and detected with an Nrf2 antibody. C3G caused a ten-fold increase in Nrf2 nuclear translocation and a 3-fold induction in Nrf2 transcription activity (FIG. 5A).

Since Nrf2 regulates the expression of the cytoprotective genes, including heme oxygenase-1, glutathione *S*-transferase (GST), and NAD(P)H:quinone
20 oxidoreductase (NQO1), the effect of C3G on GST enzymatic activity in JB6 cells was also determined. Briefly, total GST activity of the cytosolic extracts was measured spectrophotometrically as described previously using CDNB as the substrate (Habig *et al.*, *J. Biol. Chem.*, 249:7130-7139, 1974). JB6 cells were incubated with C3G for 18 hours. The cells were washed and then lysed with 200 µl
25 lysis buffer for 30 minutes. Cytosolic protein (45 µg) was added to 800 µl of reaction mixture containing 100 mM KH₂PO₄ (pH 6.5) and 1 mM glutathione. The reaction was initiated by adding 1 mM CDNB, and the formation of thioether at 5 minutes was measured at 340 nm. Total enzymatic activity of GST was expressed as nmol/min/mg protein. C3G induced a 2-fold and 3-fold induction in GST activity
30 (FIG. 5B).

This Example demonstrates that C3G is an excellent natural antioxidant, which is more powerful than ascorbate.

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Example 8**C3G INHIBITS UVB- OR TPA-INDUCED ACTIVATION OF MARKERS OF CELL MIGRATION IN A HUMAN LUNG CARCINOMA CELL LINE**

5 Recent studies have demonstrated that MAPKs, including JNK, p38, and ERK, play roles in cell migration. JNK, for example, regulates cell migration by phosphorylating paxillin, Jun, and microtubule-associated proteins. Studies of p38 show that this MAPK modulates migration by phosphorylating MAPK-activated protein kinase 2/3 (MAPKAP 2/3), which appears to be important for directionality
10 of migration. ERK is believed to govern cell movement by phosphorylating myosin light chain kinase (MLCK), calpain or focal adhesion kinase (FAK). Therefore, the different kinases in the MAPK family all seem able to regulate cell migration but by distinct mechanisms (Huang *et al.*, *J. Cell. Sci.*, 117(Pt 20):4619-4628, 2004; Huang *et al.*, *Nature*, 424(6945):219-223, 2003). Thus, inhibitory effects of C3G on JNK,
15 p38, and ERK may contribute to the suppression by C3G of tumor cell growth and metastasis. This Example demonstrates that C3G inhibits activation of biological markers of tumor cell migration in a human lung carcinoma cell line (A549 cells).

 Antibodies specific for phosphorylated ERKs, JNKs, and p38 kinase were obtained from New England Biolabs (Beverly, MA). Western blots for these
20 phospho-proteins were carried out in conformance with the manufacturer's instructions. The same blots used for detection of phospho-MAPK proteins were also probed with non-phospho-specific control antibodies provided by the manufacturer to normalize the amounts of phosphorylated ERKs, JNKs, and p38 kinase proteins.

 As shown in FIGs. 6A and 6B, C3G inhibited UVB- and TPA-induced
25 MAPKs activation in A549 cells.

 While this disclosure has been described with an emphasis upon particular embodiments, it will be obvious to those of ordinary skill in the art that variations of the particular embodiments may be used and it is intended that the disclosure may be
30 practiced otherwise than as specifically described herein. Accordingly, this disclosure includes all modifications encompassed within the spirit and scope of the disclosure as defined by the following claims:

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CLAIMS

1. A method for inhibiting a neoplasm of an exposed body surface in a subject, comprising administering a therapeutically effective amount of
5 cyanidin-3-glucoside to a subject, thereby preventing or treating the neoplasm in the subject.
2. The method of claim 1, wherein the exposed body surface is skin or cervix.
10
3. The method of claim 2, wherein the exposed body surface is skin.
4. The method of claim 1, wherein administering the cyanidin-3-glucoside comprises topical application to the exposed body surface of
15 the subject.
5. The method of claim 1, wherein the neoplasm is a papilloma, basal cell carcinoma, squamous cell carcinoma, melanoma, cutaneous T-cell lymphoma (mycosis fungoides), apocrine carcinoma of the skin, or Merkel cell carcinoma.
20
6. The method of claim 1, wherein the neoplasm is an environmentally induced neoplasm.
7. The method of claim 6, wherein the neoplasm is a chemically
25 induced neoplasm.
8. The method of claim 7, wherein the chemically induced neoplasm is a TPA-induced neoplasm.
9. The method of claim 1, wherein the method is a method of inhibiting
30 a neoplasm of an exposed body surface in a subject, and the method comprises topically applying the cyanidin-3-glucoside to the exposed body surface prior to

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exposure of the exposed body surface to an agent that promotes development of the neoplasm.

10. The method of claim 9, wherein the exposed body surface is skin.

5

11. The method of claim 10, wherein the agent that promotes development of the neoplasm is ultraviolet radiation and the cyanidin-3-glucoside is applied prior to ultraviolet radiation exposure.

10 12. The method of claim 11, wherein the cyanidin-3-glucoside is included in a topical composition that further comprises an agent capable of blocking ultraviolet radiation.

13. The method of claim 12, wherein the agent capable of blocking
15 ultraviolet radiation comprises para-aminobenzoate (PABA), ethylhexyl methoxycinnamate, DEA methoxycinnamate, padimate O, ethylhexyl salicylate, homosalate, TEA salicylate, oxybenzone, dioxybenzone, sulisobenzene, avobenzone, octocrylene, titanium dioxide, zinc oxide or menthyl anthranilate, or combinations thereof.

20

14. The method of claim 4, wherein the exposed body surface is the cervix.

15. The method of claim 14, wherein the neoplasm is a papilloma.

25

16. The method of claim 15, wherein the cervical papilloma is induced by a human papillomavirus.

17. The method of claim 1, wherein the subject is a mammal.

30

18. The method of claim 17, wherein the subject is a human.

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19. The method of claim 1, wherein treating the neoplasm comprises inhibiting metastasis of the neoplasm.

20. The method of claim 1, wherein treating the neoplasm comprises
5 reducing the size of the neoplasm.

21. The method of claim 4, wherein a therapeutically effective amount comprises from about 0.2% to about 1% cyanidin-3-glucoside (w/w).

10 22. A method of inhibiting metastasis of a malignant cell comprising contacting at least one malignant cell with an amount of cyanidin-3-glucoside sufficient to inhibit metastasis.

23. The method of claim 22, wherein the malignant cell originates from a
15 lung neoplasm or a skin neoplasm.

24. The method of claim 22, wherein an amount sufficient to inhibit metastasis comprises from about 8 to about 10 mg/kg cyanidin-3-glucoside.

20 25. The method of claim 24, wherein contacting at least one malignant cell with an amount of cyanidin-3-glucoside sufficient to inhibit metastasis comprises administering the cyanidin-3-glucoside to a subject.

26. The method of claim 25, wherein administering cyanidin-3-glucoside
25 to the subject comprising intrathecal administration, intradermal administration, intramuscular administration, intraperitoneal (ip) administration, intravenous (iv) administration, subcutaneous administration, intranasal administration, epidural administration, or oral administration, or combinations thereof.

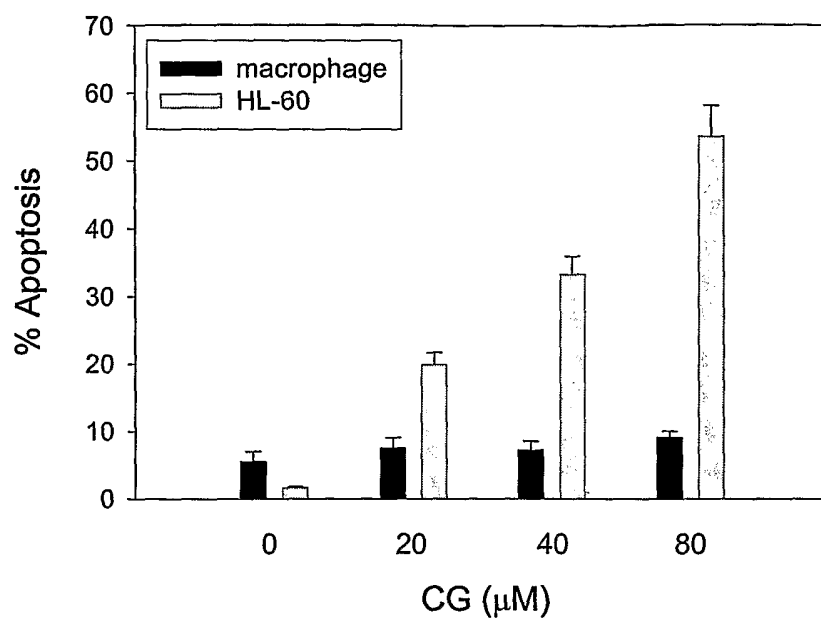
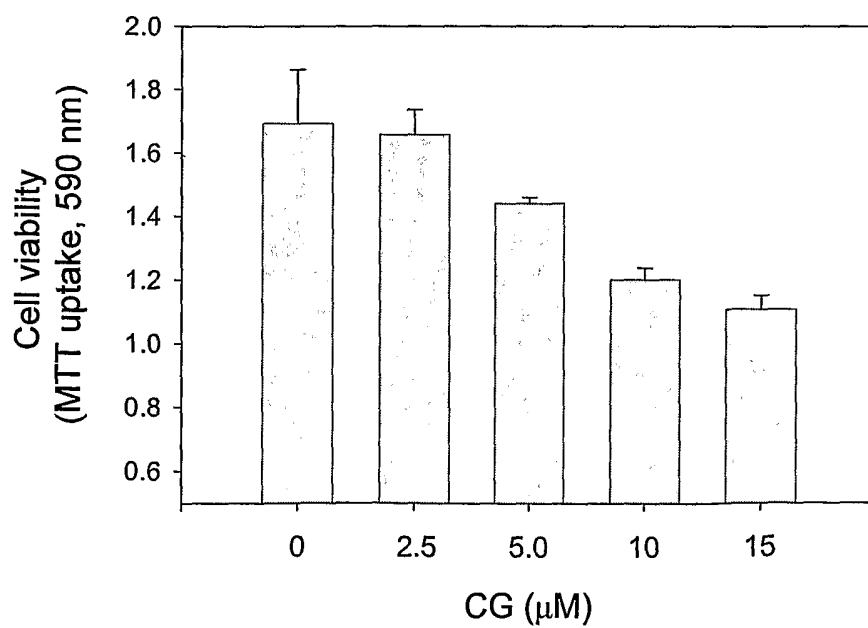
30 27. A topical composition comprising cyanidin-3-glucoside and an agent capable of blocking ultraviolet radiation.

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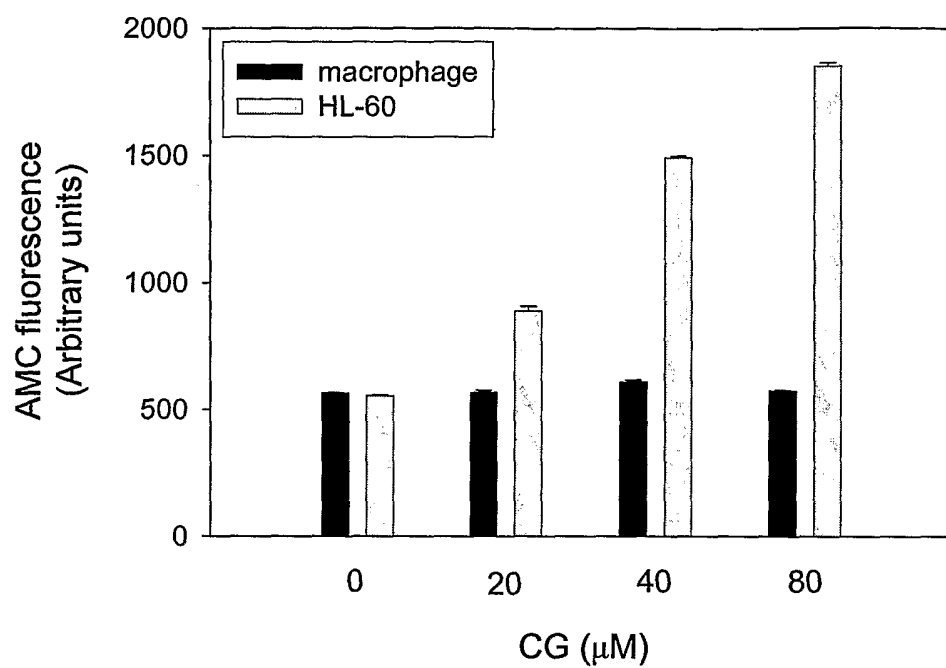
28. The topical composition of claim 27, wherein the agent capable of blocking ultraviolet radiation is para-aminobenzoate (PABA), ethylhexyl methoxycinnamate, DEA methoxycinnamate, padimate O, ethylhexyl salicylate, homosalate, TEA salicylate, oxybenzone, dioxybenzone, sulisobenzene, 5 avobenzene, octocrylene, titanium dioxide, zinc oxide or menthyl anthranilate, or combinations thereof.

29. The topical composition of claim 27 further comprising an emollient or antioxidant, or combination thereof.

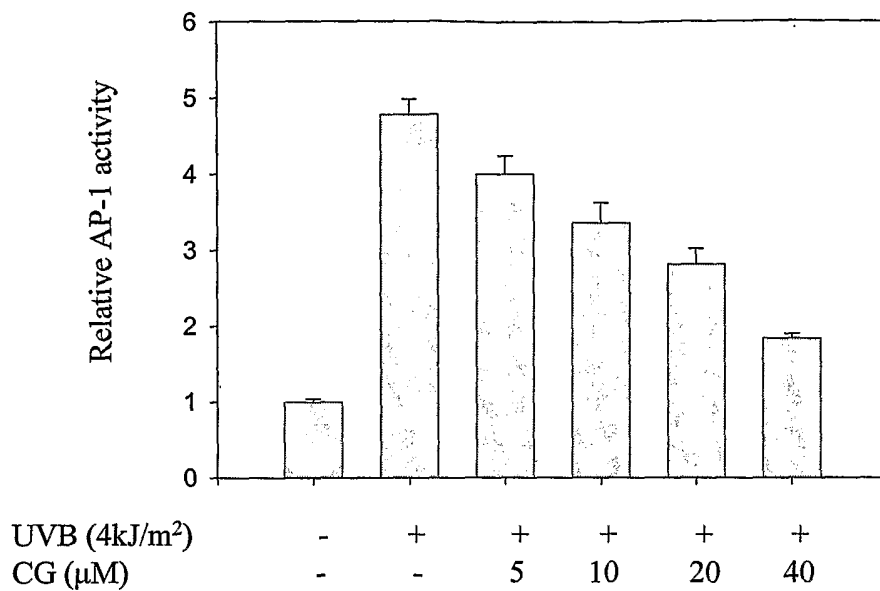
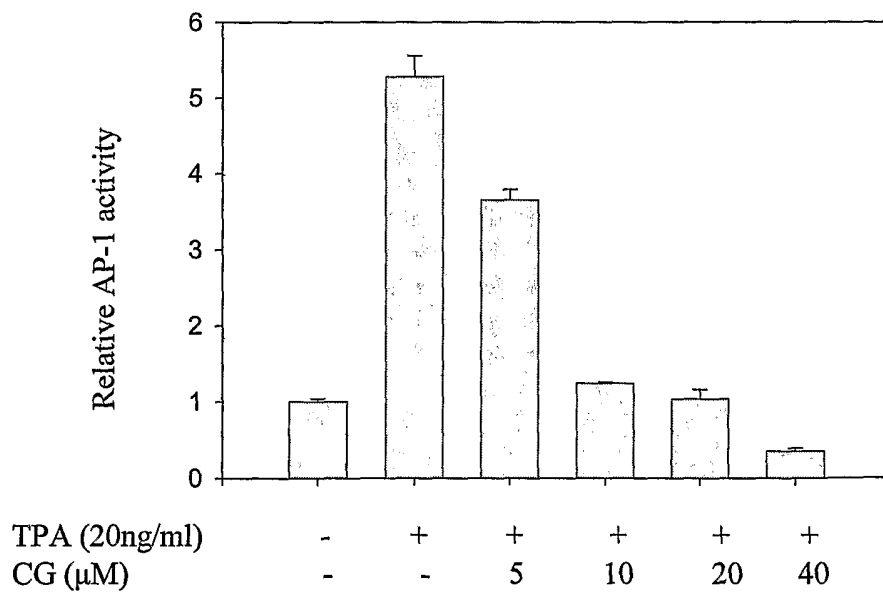
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FIG. 1A**FIG. 1B**

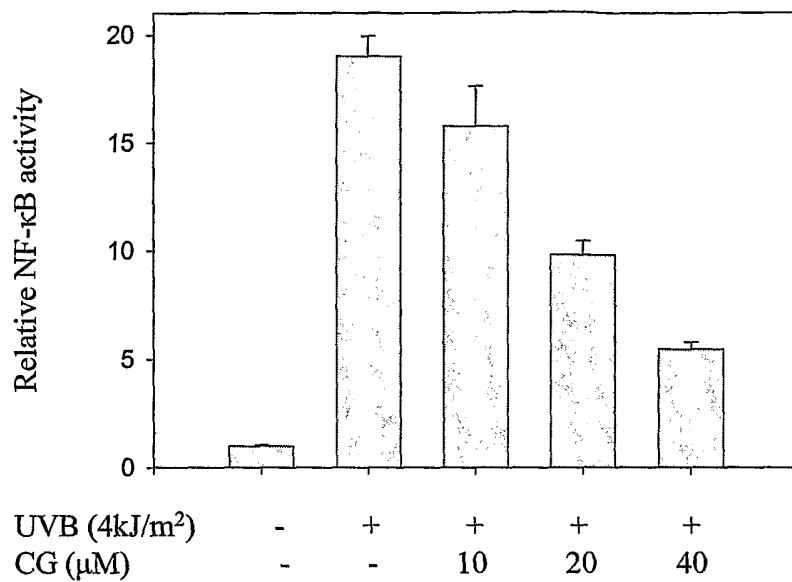
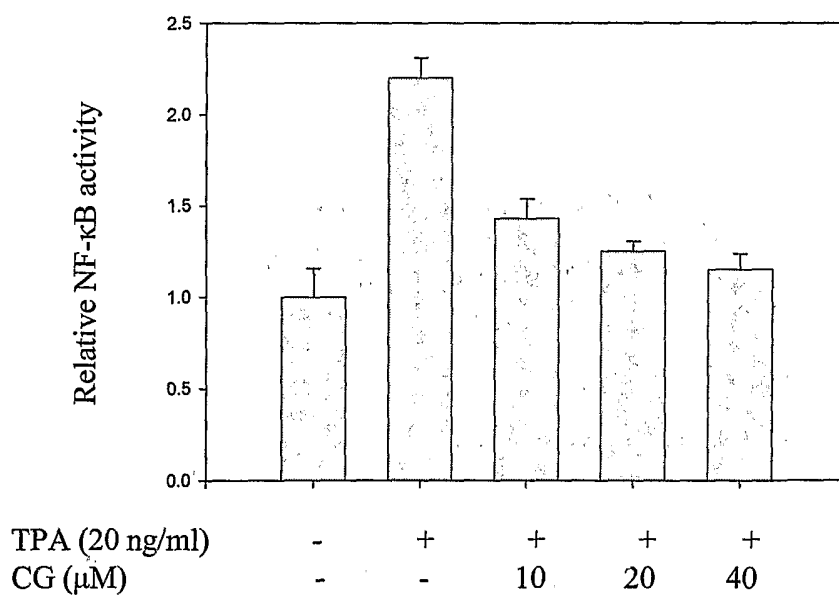
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FIG. 1C

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FIG. 2A**FIG. 2B**

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FIG. 2C**FIG. 2D**

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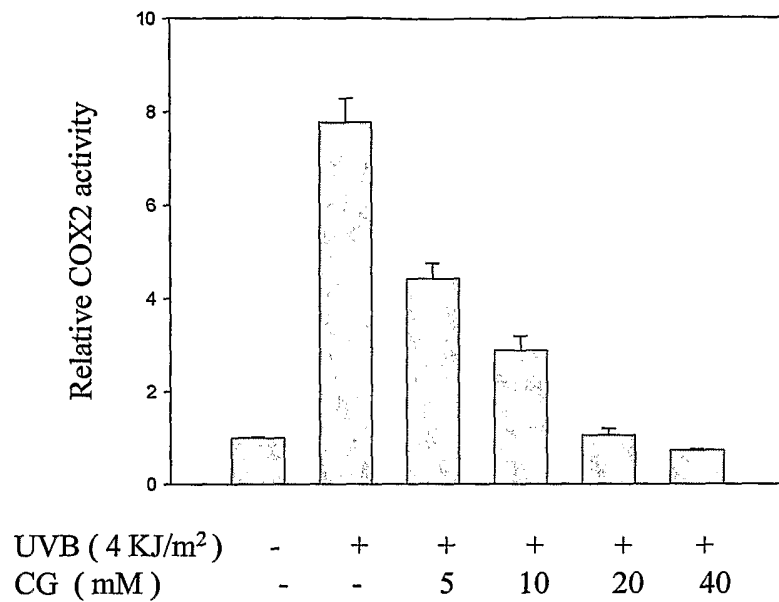
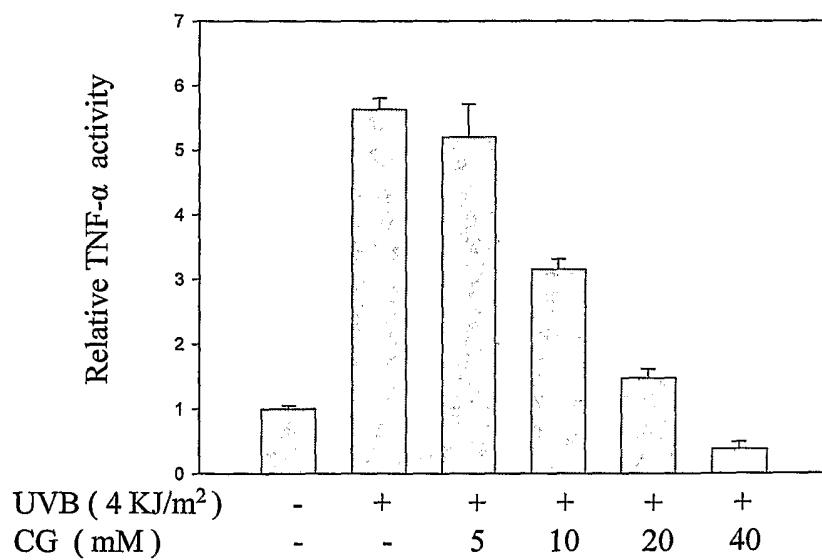
FIG. 2E**FIG. 2F**

FIG. 2G

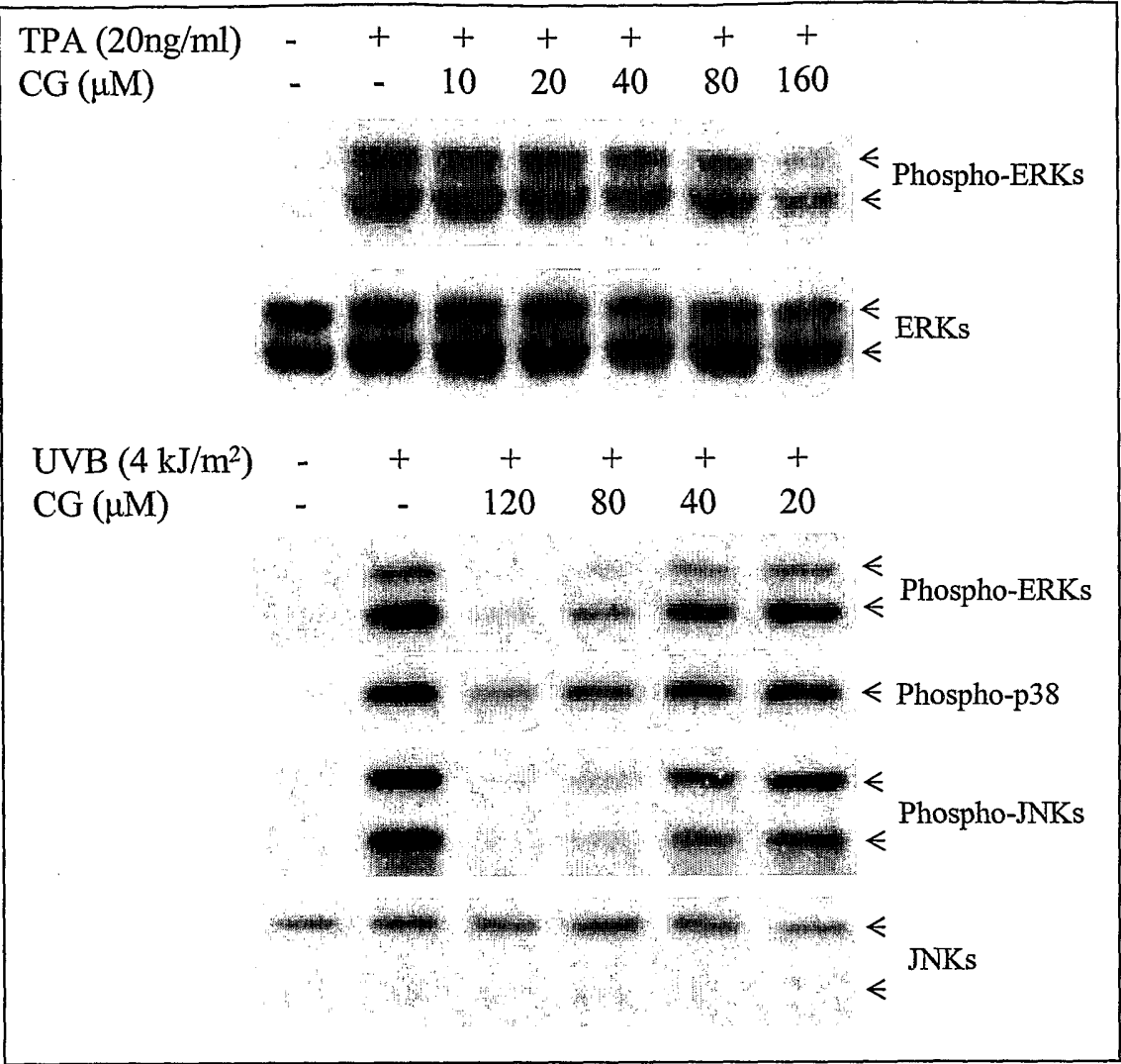
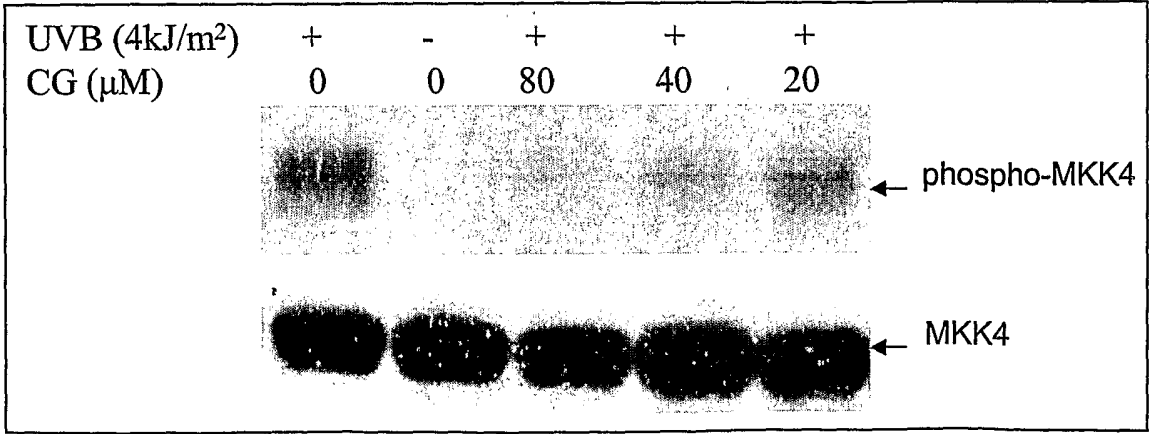
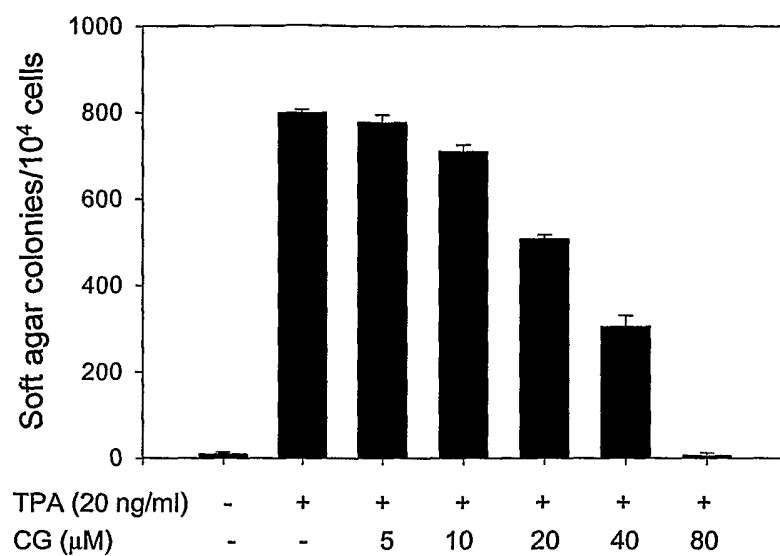
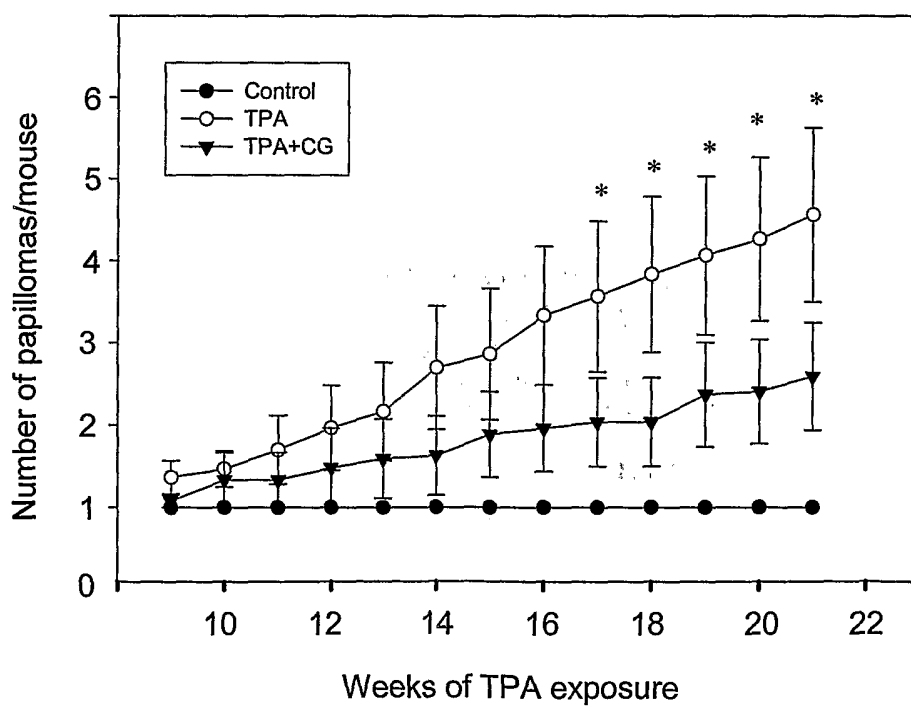


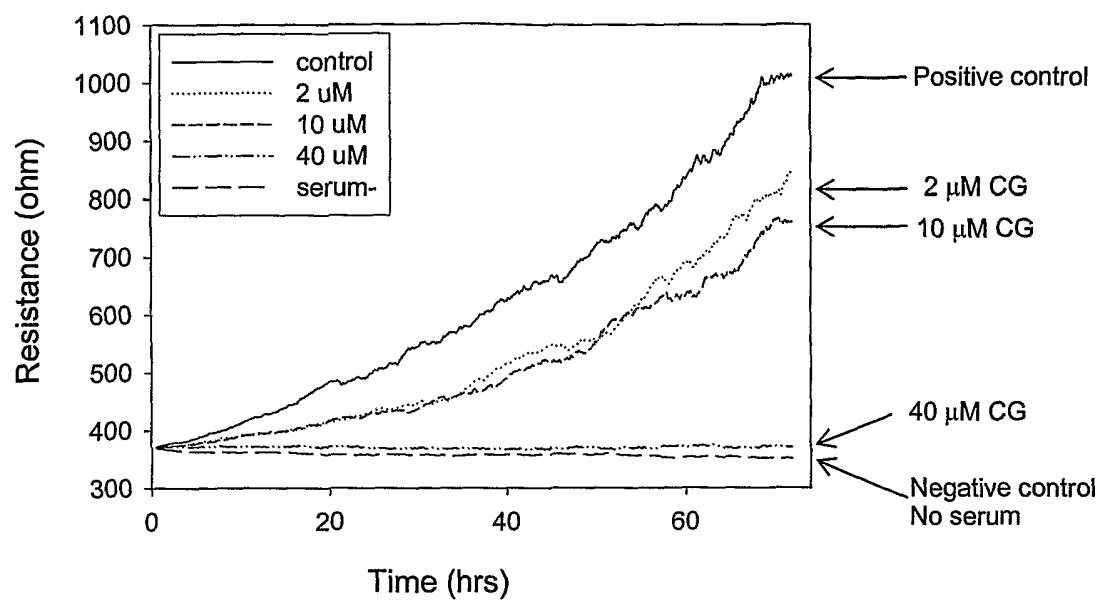
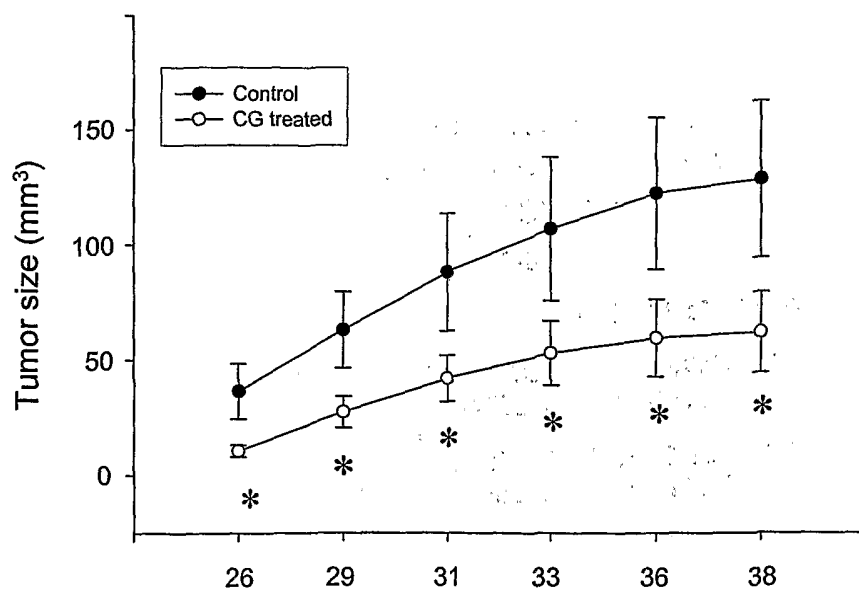
FIG. 2H



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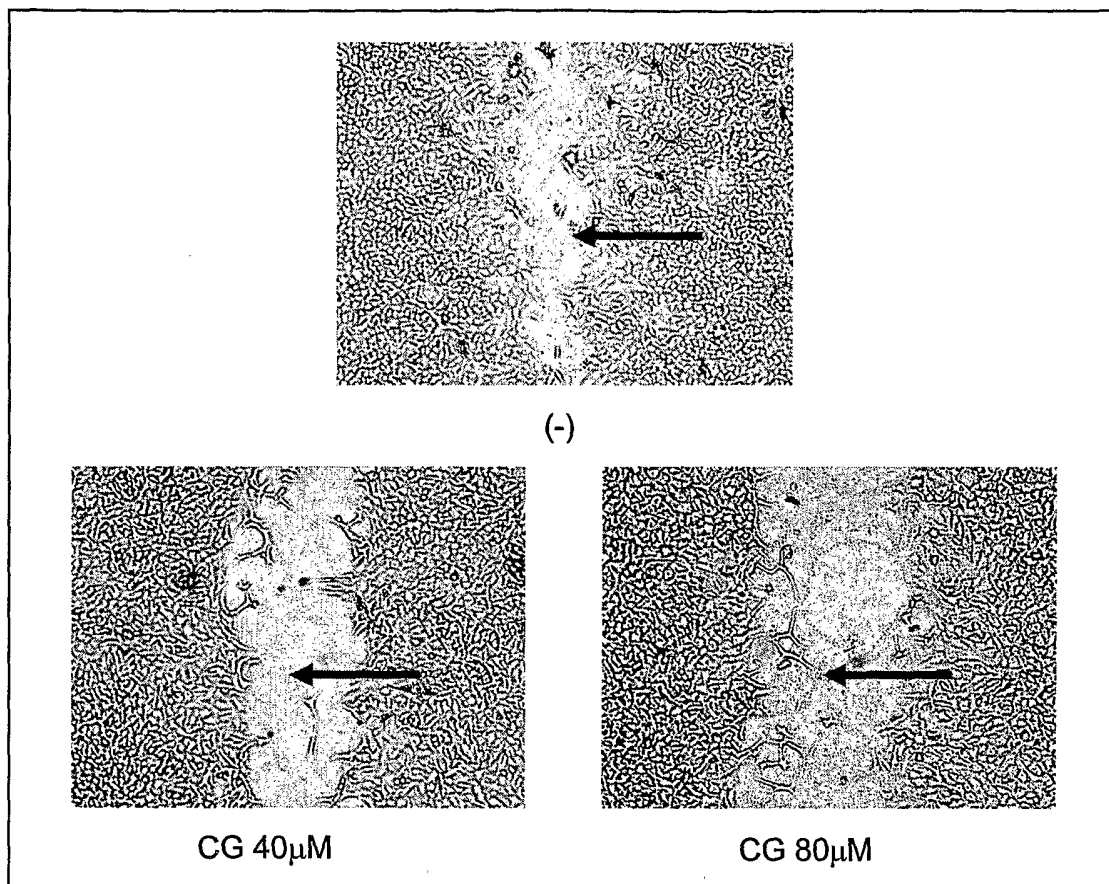
FIG. 3A**FIG. 3B**

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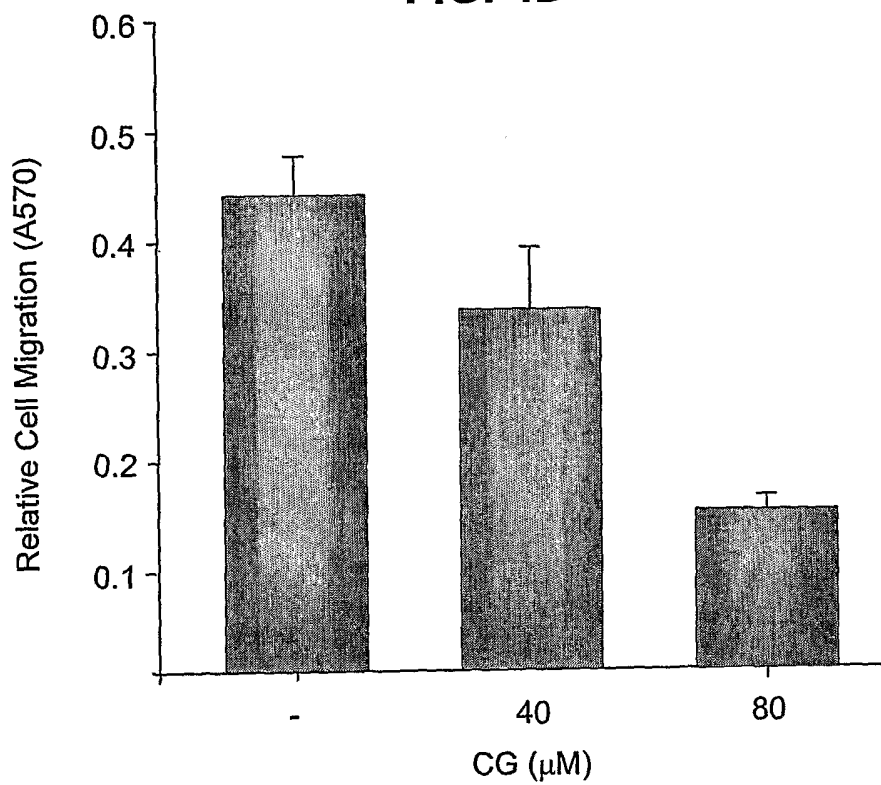
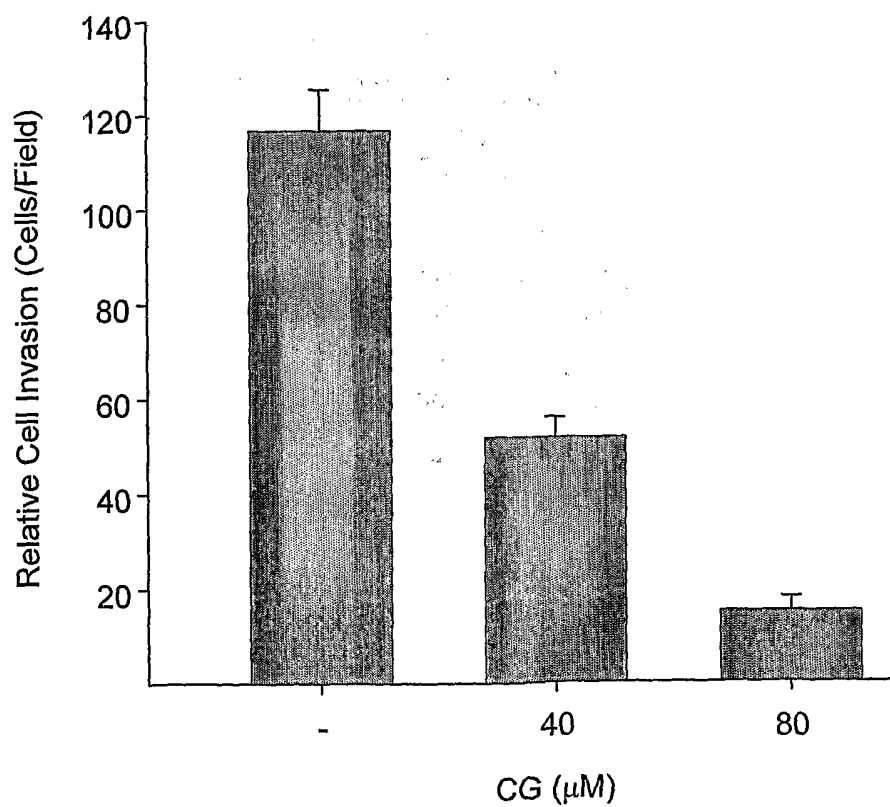
FIG. 4A**FIG. 4B**

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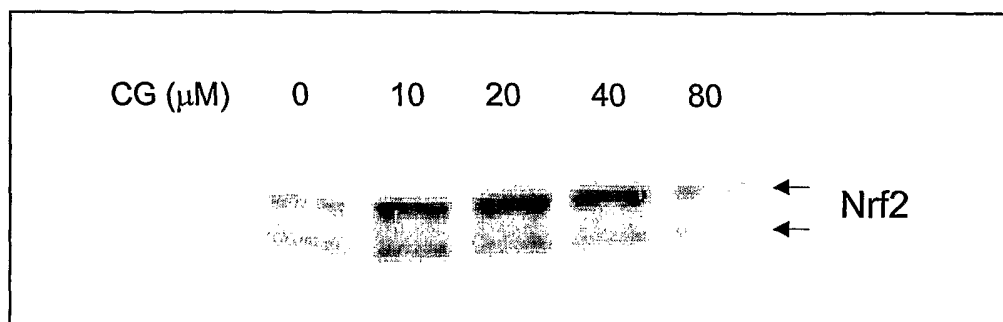
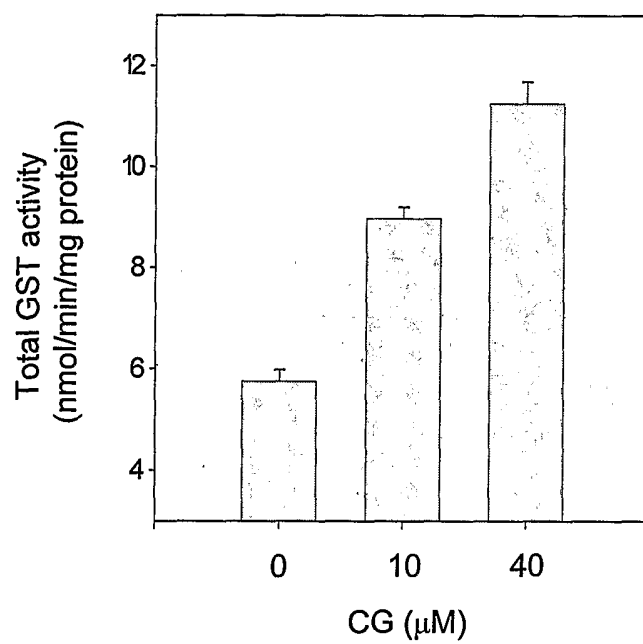
FIG. 4C



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FIG. 4D**FIG. 4E**

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FIG. 5A**FIG. 5B**

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FIG. 6A

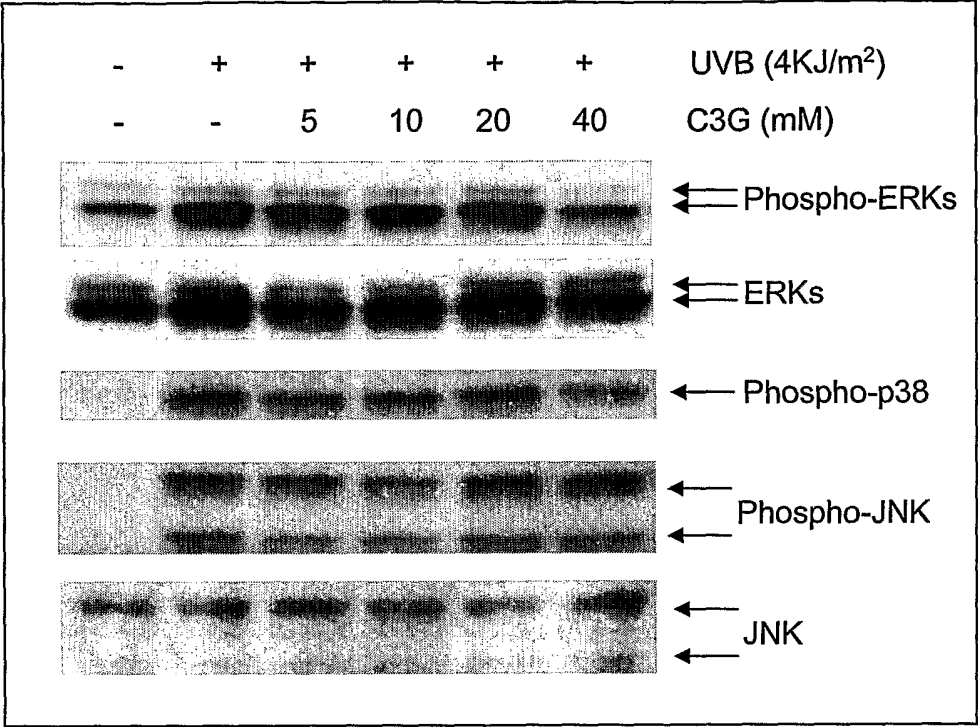


FIG. 6B

