METHODS OF TREATMENT OF KERATINOCYTE-DERIVED LESIONS

ACETONE

CAMPHOR OIL

FIG. 15

TRPV3 agonists, e.g., camphor, camphor-oil which contain monoterpenoid constituents, 2-APB and derivatives thereof arrest human keratinocyte proliferation and promote differentiation. Methods are provided for diagnosing and treating or prevent -ing keratinocyte-derived lesions, e.g., SCC, (including high-risk forms), non-melanoma skin cancers (including high-risk forms), and actinic keratosis (“AK”) by administering to an afflicted subject a therapeutically effective amount of one or more TRPV3 agonists or derivatives thereof. Pharmaceutical compositions that contain therapeutically effective amounts of one or more TRPV3 agonists or derivatives thereof are also contemplated.
METHODS OF TREATMENT OF KERATINOCYTE-DERIVED LESIONS

CROSS-REFERENCE TO RELATED APPLICATIONS


STATEMENT OF GOVERNMENTAL INTEREST

[0002] This invention was made with Government support under Contract No. NIH-NIAMS R01AR051219 awarded by the National Institutes of Health National Institute of Arthritis and Musculoskeletal and Skin Diseases. The Government has certain rights in the invention.

BACKGROUND

[0003] Squamous cell carcinoma (SCC) is the second most common form of skin cancer in the United States. A significant subset of high-risk forms of SCCs are highly invasive and metastatic. These SCCs are associated with a comparatively high risk of recurrence, resulting in significant mortality. SCC can be diagnosed by biopsy, however, current diagnostic biomarkers cannot distinguish high-risk SCCs that are likely to become metastatic from those that can be effectively treated with surgery alone. Current methods of treatment, i.e. surgery, radiotherapy, and chemotherapy, require continued monitoring due to the metastatic nature of the disease.

[0004] To date, there is no accepted system for defining high-risk SCC. The potential for advanced or aggressive disease can be attributed to a combination of tumor factors and host factors. Most high-risk tumors will have more than one risk factor present. As prognostic models have not yet been developed, it is unknown how various combinations of risk factors impact risk of recurrence or metastasis. Thus, it remains difficult to estimate these risks for an individual patient and make reasonable treatment recommendations. Development of reliable prognostic models will greatly aid treatment decisions in SCC.
SUMMARY OF THE INVENTION

[0005] In certain embodiments, methods are provided for diagnosing and treating or preventing keratinocyte-derived lesions, e.g., SCC (including high-risk forms), non-melanoma skin cancers (including high-risk forms), and actinic keratinosis ("AK") by administering to the subject a therapeutically effective amount of one or more TRPV3 agonists or derivative thereof. The TRPV3 agonist may be a monoterpene as listed in Table A herein, or the TRPV3 agonist may be camphor oil which contains other monoterpene constituents as listed in Table A herein, or the TRPV-3 agonist is 2-APB. Embodiments are also directed to pharmaceutical compositions and kits comprising one or more TRPV3 agonists, preferably formulated for topical application.

[0006] In the experiments described herein, amounts of camphor ranging from 4-8 mM were shown in vitro to reduce keratinocyte proliferation and further reduced concentrations of 2-APB (50 µM) reduced SCC tumor burden and invasiveness in organotypic human skin cultures. Camphor in acetone was also applied at concentrations from about 10%-30% to mice to stimulate the expression of a keratinocyte terminal differentiation marker and camphor oil at 20% treat SCC in vivo. In embodiments of the invention for treating or preventing SCC, (including high-risk forms) or non-melanoma cancers (including high-risk forms), or AK, the TRPV3 agonists camphor and camphor oil can be applied in amounts ranging from 0.0608-99.5% (wt/vol or vol/vol), preferably in the range of 10-50%. The amount of 2-APB was tested in concentrations of 12.5 µM to 100 µM in vitro and about 25 µM to 50 µM was found to be effective in attenuating proliferation, which corresponds to 0.000056-0.00011%. The amount of 2-APB for treating or preventing SCC, (including high-risk forms) or non-melanoma cancers (including high-risk forms), or AK in vivo ranges from about 0.00005-5%. The TRPV3 agonist(s) or derivatives thereof are preferably applied directly to the affected area, including the margins of the tumor, either before the lesions surgically removed or after the lesion is surgically removed. In some cases treatment with the TRPV3 agonist(s) or derivative thereof may eliminate the need for surgical removal of a non-aggressive, non-high risk lesion.

[0007] It has been discovered that dysregulated expression of one or more TRP channels (e.g., TRPV3, TRPC1, TRPV1, and TRPA1) in SCC and other non-melanoma cancers is a
biomarker for a high risk form of SCC and high risk non-melanoma respectively. In an embodiment, a method is provided to diagnose the high-risk form of SCC (or high risk non-melanoma) in a patient by determining if a biopsy of the SCC (or high risk non-melanoma), has a significantly higher or a significantly lower level of mRNA encoding one or more TRP channels (e.g., TRPV3, TRPC1, TRPV1, and TRPA1) compared to the respective mRNA levels in a control sample of normal tissue. Thus, in some embodiments, methods comprise obtaining a biopsy from a subject suspected of having or diagnosed as having SCC or non-melanoma cancers, and obtaining a control biopsy—either from a normal patient not afflicted with any cancer and not having an endogenous TRP mutation, or from a matched-sample from a non-involved, normal area from the cancer subject. The levels of mRNA encoding TRPV3, TRPC1, TRPV1, and TRPA1 in the cancer biopsy and in the control biopsy are then determined, and if the level of mRNA encoding one or more of the following: TRPV3, TRPC1, TRPV1, and TRPA1 in the cancer biopsy is significantly changed (i.e., is either significantly higher or significantly lower) than the corresponding level in the control biopsy, then diagnosing that the patient has a high-risk form of cancer: i.e., either high risk SCC or high risk non-melanoma. Once the diagnosis of high-risk SCC or high risk non-melanoma is made, then it is determined that the subject is in need of more aggressive treatment (e.g., Mohs surgery followed by chemotherapy and radiation). Or treatment may be the direct application of a TRPV3 agonist to the squamous cell carcinoma or high-risk non-melanoma cancer before and after it is removed. High-risk subjects can be immunocompromised patients (e.g., organ transplant patients.)

[0008] Finally, embodiments are directed to pharmaceutical compositions and kits containing them that contain therapeutically effective amounts of one or more TRPV3 agonists or derivatives thereof. The TRPV3 agonist may be a monoterpene (e.g., camphor, (+)-Borneol, (-)-Isopinocampeol, (-)-Fenchone, (-)-Trans-pinocarveol, Isoborneol, (+)-Camphorquinone, (-)-a-Thujone, alpha-pinene oxide, 1, 8-Cineole, (-)-alpha-Pinene, Isobornyl acetate, 6-tert-butyl-m-cresol, Carvacrol, Dihydrocarveol, Thymol, /?/-xylanol, Kreosol, Propofol, p-cymene, Carvacrol methylether, Dihydrocarveol, (-)-Carveol, and (-)-Isopulegol, (-) methol, (-)-Carvone, (+)-Dihydrocarvone, (-)-Menthone, (+)-Limonene, Terpineol, (+)-Linalool Geraniol, l-Isopropyl-4-methyl-bicyclo[3.1.0] hexan-4-ol, (-)-alpha-Bisabolol, and mugetanol), or it may be camphor- oil which contains other monoterpene constituents, or the TRPV-3 agonist is
2-APB, for treatment or prevention of keratinocyte-derived lesions, e.g., SCC (including high-risk forms), non-melanoma cancer (including high-risk forms), or AK.

[0009] Other embodiments are directed to sunscreens containing one or more of these TRPV3 agonists or derivatives thereof in amounts that reduce or prevent abnormal keratinocyte proliferation. Sunscreens comprising the TRPV3 agonists or derivatives thereof are recommended for individuals who are routinely exposed to the sun and should especially be applied routinely to lips, ears and face. Since SCC is very prevalent in human populations, and is typically on sun-exposed skin, anyone with significant accumulated sun exposure over his or her lifetime is at some risk of SCC. Dosage ranges of the TRPV3 agonists or derivatives thereof that can be used in sunscreens are as described herein. In an embodiment, the pharmaceutical compositions may be formulated into liposomes for topical application or microinjection and sold commercially in kits.

[0010] Other embodiments may include: (i) methods for treating squamous cell carcinoma or actinic keratosis in a patient in need thereof, (ii) for slowing the progression of benign tumors to squamous cell carcinoma or actinic keratosis in a patient in need thereof, (iii) promoting regression of pre-malignant skin tumors in a patient in need thereof, (iv) attenuating malignant squamous cell carcinoma conversion in a patient in need thereof, or (v) preventing metastasis into an area around the tumor are also contemplated comprising contacting one or more skin lesions with the above-described pharmaceutical composition.

[0011] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, appended claims, and accompanying figures.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0012] The following figures form part of the present specification and are included to further demonstrate certain embodiments of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0013] Figure 1 (FIG. 1). Cytoplasmic calcium signals elicited by TRP-channel agonists are potentiated in differentiating human keratinocytes. Responses to TRP-channel agonists (A-D) and gene expression levels (E) were compared in normal human epidermal
keratinocytes cultured for 2-3 days in 0.06 mM Ca^{2+} (Growth; green) or in (Differentiated; blue). (A-D) Live-cell Ca^{2+} imaging. (A) Pseudocolor images depict fura-2 ratios of human keratinocytes at rest (left) and during acute application of saturating concentrations of two TRP channel agonists (10 mM Camphor; middle) and TRPV4 (3 µM 4aPDD; right). (B) The percent increases in fluorescence ratio are plotted for representative individual keratinocytes (colored lines). Responding cells were identified as those showing a 20% increase in fluorescence ratio during agonist application. Symbols depict time points in (A). (C) Quantification of TRP-agonist-evoked responses (N=3 experiments with >300 cells per replicate, X^2<0.0001). (D) Responses to structurally unrelated TRPV3 agonists, camphor and 2-APB, were larger in differentiated human keratinocytes (N=3 experiments with >640 cells per replicate; symbol *P<0.05; two-way ANOVA). (E) Quantitative PCR demonstrated that TRPV3 and keratinocyte differentiation markers (keratin-1, KRT1; loricrin, LOR; and filaggrin; FLG) were upregulated upon differentiation. Fold increase in transcript levels compared with growth conditions is plotted (N=3 experiments; ***P<0.0001, *P<0.05, Student's t test).

[0014] Figure 2 (FIG. 2) TRP channel responses and expression in human keratinocytes. (A) Fura-2 responses to TRPV1 specific agonist, 1µM Capsaicin, and TRPA1 agonist, 300 µM mustard oil, in human keratinocytes (Capsaicin=2.3+/− 2.6%, Mustard Oil=0.6+/− 1.2%; N=3 experiments). These responses demonstrate that responses to TRPV1 and TRPA1 agonists are not significantly different from zero. Thus, these TRP channels are functionally expressed at lower levels than TRPV3 in human keratinocytes in vitro. (B) Quantitative PCR demonstrated that TRPV1 and TRPC1 were upregulated upon differentiation. TRPA1 was only amplified in one out of three experimental replicates, demonstrating that this transcript is expressed at low levels compared with TRPV3, TRPV1 and TRPC1. TRPV3 expression is plotted for reference. Expression level relative to GAPDH levels is plotted for both growth and differentiation conditions (N=3 experiments; **P<0.0004, *P<0.05, Student's t test).

[0015] Figure 3. (FIG.3). Constitutive activation of TRPV3 arrests human keratinocyte proliferation and promotes differentiation. (A)-(B) Quantification of DAPI-labeled keratinocytes cultured in range of 2-APB concentrations. Brightfield images (left) correspond to concentrations plotted at right. Percent change from vehicle-treated control cells is plotted (N=5 experiments, **P≤0.006, Student's t test). (C) Proliferation assays (45-min EdU pulse)
after 24 hours treatment with vehicle, 12.5 µM, or 50 µM 2-APB (top panel, N=5 independent experiments, *P<0.05, **P<0.0001, Student's two-tail t test) or after 24 h treatment with vehicle, or camphor at the indicated concentrations (lower panel; N=3 independent experiments, **P<0.006, Student's two-tail t test). (D) Gene expression levels compared by qPCR (LOR, FLG, Involucrin: IVL, Transglutaminate 3: TGM3) in keratinocytes treated with vehicle (1% ethanol) or 50 µM 2-APB. Fold increase in transcript levels in 2-APB-treated compared with vehicle- treated keratinocyte is plotted (N=3 independent experiments; *P<0.02, **P<0.006, ***P<0.0001, Student's t test). (E) Organotypic 3D human skin equivalents treated with vehicle (top panel) or 50 µM 2-APB (bottom panel). Mean number of nucleated epidermal cell layers is plotted (n=26-28 sections from two grafts per treatment, E=epidermal layer, D=dermis. ***P<0.0001). (F) Mean number of nucleated epidermal cell layers is plotted (n=26-28 sections from two grafts per treatment, E=epidermal layer, D=dermis, ***P<0.0001).

[0016] Figure 4. (FIG.4). Inhibition of TRPV1 does not alter camphor effects on human keratinocytes. (A) Quantification of DAPI-labeled keratinocytes cultured in range of camphor concentrations with or without 0.5-1 µM AMG 9810. Percent change from vehicle-treated control cells is plotted (N=3 experiments; Two-way ANOVA, camphor P=0.0004, AMG-9810 P=0.56). (B) Proliferation assays (45-min EdU pulse) after 24 hours treatment with vehicle, 2-8 mM, camphor with or without 0.5-1 µM AMG 9810. Percent EdU positive cells are plotted (N=3 experiments; Two-way ANOVA, camphor P=0.0004, AMG-9810 P=0.57). These data indicate that camphor attenuates keratinocyte proliferation by acting on at target other than TRPV1.

[0017] Figure 5. (FIG. 5.) Epidermal TRP channels are potential therapeutic targets for the treatment of human SCC. (A) TRPV3 expression levels assessed by qPCR in high-risk SCC biopsies (purple patterns) and normal adult skin. Fold increase compared with normal adult skin is plotted (black; N=4 replicates per sample, ¥ P<0.05, ¥¥ P<0.005, Student's t test). Plots depict means+SDs. (B) Live-cell calcium imaging of TRP-channel activation in two SCC cell lines. Fura-2 fluorescence ratios of SCC cells at rest and during camphor application (10 mM) are shown. Right: quantification of camphor-evoked responses (N>200 cells per SCC cell line; ¥ P<0.05, Student's t test). (C) Transcript levels monitored by qPCR in human SCC-derived cell lines, SCC-13 (purple) and SCC-39 (blue), and normal keratinocytes (black).
Fold increase compared with normal keratinocytes is plotted (N=4 replicates; *P<0.0001, # P<0.005, ¥ P<0.05, Student's t test). Plots depict means+SDs. (D) Organotypic human skin equivalents with SCC cells were treated with vehicle (SCC-39; upper right) or 50 µM 2-APB (SCC-39; lower right) for 14 days. (E) Number of invading cells per 10X field (upper) and SCC layer thickness (lower) were quantified for SCC-39 (purple) and SCC-13 (blue) organotypic cultures (3 rafts per treatment, T=tumor, D=dermis, # P<0.005 for treatment, two-way ANOVA).

Figure 6. (FIG. 6). TRP channel and SCC biomarker expression in SCC biopsies. (A) TRPV1, (B) TRPA1, (C) TRPC1, (D) CCND1, and (E) EGFR expression levels assessed by qPCR in high-risk SCC biopsies (purple patterns) and normal adult skin (black). Fold increase compared with normal adult skin is plotted (N=4 replicates per sample, *P<0.0001, Student's t test). Plots depict Means +/- SDs.

Figure 7. (FIG. 7). TRP channel expression in SCC cell lines. TRPV1, TRPA1 and TRPC1 expression levels assessed by qPCR in SCC cell lines SCC-13 (purple), SCC-39 (blue) and normal human keratinocytes (black). Fold increase compared with normal adult skin is plotted (N=4 replicates per sample, **P<0.009, ***P<0.0001, Student's t test). Plots depict Means +/- SDs.

Figure 8. (FIG. 8). SCC skin equivalents show reduced tumor thickness when treated with 2-APB. Micrographs show organotypic human skin equivalents with (A) SCC-73, (B) SCC-39, and (C) SCC-13 cells treated with vehicle (upper) or 50 µM 2-APB (lower) for 14 days. These three SCC cell lines were derived from three independent, de-identified, human high-risk SCC tumors.

Figure 9. (FIG. 9). SCC-13 skin equivalents show reduced tumor thickness when treated with 2-APB. Micrographs show organotypic human skin equivalents with SCC-13 cells treated with vehicle (A) or 50 µM 2-APB (B) for 14 days.

Figure 10. (FIG. 10). Two-stage chemical carcinogenesis model (DMBA-TPA). A two-stage chemical carcinogenesis model (DMBA-TPA) was used to induce benign papillomas and SCCs in adult female mice. Mice were randomly assigned to treatment groups control (acetone) or 20% camphor oil and (N=12 mice per group) matched for lesion burden immediately prior to the start of treatment (week 0). Lesions were quantified and cohorts were photographed weekly beginning two weeks before treatment.
Figure 11. (FIG. 11). Total Number of Malignant SCCs in mice treated with 20% camphor oil or vehicle. Groups were compared with two-way ANOVA followed by Bonferroni post hoc analysis to assess significant differences between treatment groups at each time point (N=12 mice per group; *P<0.05; **P<0.01). Significant effects included treatment group: F(15,265)=101.40, P<0.0001; treatment duration: F(15,265)=5.16, P<0.0001; interaction: F(15,265)=2.30, P=0.004.

Figure 12. (FIG. 12). Example of regression of benign papillomas in a camphor oil-treated mouse compared with an acetone (vehicle)-treated control animal. At week 0 in the camphor oil-treated mouse, 10 tumors and at week 7, 1 tumor compared at week 0 in the vehicle-treated control, 13 tumors and at week 7, 11 tumors.

Figure 13. (FIG. 13). Total number of malignant SCCs in mice treated with 20% camphor oil or vehicle alone. Curves show Boltzmann fits (R²>0.93 for each fit), which differed significantly (P<0.0001, extra Sum of Squares F test). Two-way ANOVA showed a significant effect of treatment group [F(1,15)=10.20, P=0.006] and treatment duration [F(15,15)=2.74, P=0.030] (N=12 mice per group).

Figure 14. (FIG. 14). Regression of early-stage SCC and papilloma in a camphor-oil treated mouse. An advanced SCC (bracket), which appeared by treatment day 9, progressed to an experimental endpoint by 38 days of treatment. An early-stage SCC (arrow) and a papilloma (arrowhead) on this mouse appeared to regress between treatment day 37 and 38.

Figure 15. (FIG. 15). Hematoxylin and eosin (H&E) staining of paraffin sections from SCC tumors in control and camphor-oil treatment groups. Advanced SCCs showed neoplastic keratinocytes surrounding muscle tissue (arrowheads), indicating an invasive lesion. A regressed SCC tumor from the camphor-oil group (right) showed an intact muscle layer (brackets). The middle and right panels are imagines of the advanced and regressed SCCs in Figure 14.

Figure 16. (FIG. 16). Tumor incidence in mice treated with camphor oil or vehicle. Tumor incidence remained at 100% in control mice but decreased to as low as 50% in camphor oil-treated mice (N=12 mice per group). A two-way ANOVA showed a highly significant effect of treatment group on tumor incidence [F(1,15)=14.40, P=0.0018]. The effect of treatment day was not significant [F(15,15)=1.0, NS].
Figure 17. (FIG. 17). Time to experimental endpoint in mice treated with camphor oil or vehicle alone (N=12 mice per group). After 13 weeks of treatment, 75% of control mice and 50% of camphor oil-treated mice reached an experimental endpoint. But Kaplan-Meier survival analysis indicated that endpoint curves did not differ significantly between treatment groups.

Figure 18. (FIG. 18). Topical camphor upregulates levels of a keratinocytes terminal differentiation marker (K10) in vivo. FVB mice (adult females; N=3-4) were treated twice per day for five days with camphor diluted in vehicle (acetone) at the indicated concentrations. Protein levels were assayed by Western blotting, normalized to beta-tubulin levels, and means were compared with Student's t tests (one-way unpaired). P values for pairwise comparisons are indicated in the figure.

DETAILED DESCRIPTION

1. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference.

Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics, protein, and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. 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 unless otherwise indicated. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992, and Supplements to 2002); Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990); Principles of Neural Science, 4th ed., Eric R. Kandel,
James H. Schwart, Thomas M. Jessell editors. McGraw-Hill/ Appleton & Lange: New York, N. (2000). Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

[0033] The term actinic keratosis (also called "solar keratosis" and "senile keratosis," herein "AK"), as used herein, means a premalignant condition of thick, scaly, or crusty patches of skin. It is more common in fair-skinned people and it is associated with those who are frequently exposed to the sun, as it is usually accompanied by solar damage. AK is potentially pre-cancerous, since some progress to squamous cell carcinoma, so treatment is recommended. Untreated lesions have up to 20% risk of progression to squamous cell carcinoma. People who take immunosuppressive drugs, such as organ transplant patients, are 250 times more likely to develop actinic keratosis that may lead to skin cancer.

[0034] The term "activating a transient receptor potential vanilloid ion channel" ("TRPV channel") as used herein, means increasing the proportion of receptors in an ion-conducting confirmation, which is expected to increase cation transport into the cell. This has the effect of reducing or arresting proliferation or normal and cancerous (e.g., SCC cells) human keratinocytes.

[0035] The term, "active agent" as used herein, collectively refers to TRPV3 agonists as disclosed herein, and derivatives thereof (e.g., monoterpenes such as those listed in Table A, such as camphor, camphor oil, which contains monoterpenic constituents, and 2-APB for treatment or prevention of keratinocyte-derived lesions, e.g., SCC (including high-risk forms), non-melanoma skin cancers (including high-risk forms), and AK.

[0036] The term "enumerated disease" as used herein means any keratinocyte-derived lesion including SCC (which encompasses the high risk form), non-melanoma cancer (which encompasses the high risk form), and AK.

[0037] As used herein, "administering" an active agent may be performed using any of the various methods or delivery systems well known to those skilled in the art. The preferred method for administering camphor or derivatives thereof, camphor oil which contains other monoterpane constituents, or 2-APB at a dose that might be toxic systemically, is topical/transdermal administration directly to the keratinocyte-derived lesion. The administering can also be performed, for example, orally, parenterally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally,
transbuccally, intranasally, liposomally, via inhalation, vaginally, intraocularly, via local
delivery, subcutaneously, intraadiposally, intraarticularly, intrathecally, into a cerebral
ventricle, intraventricularly, intratumorally, into cerebral parenchyma or intraparenchymally
or microinjection.

[0038] As used herein "agonist" refers to molecules or compounds which mimic the action of a
"native" or "natural" compound that activates one or more of the ion channels TRPV3, TRPV1,
TRPA1, or TRPC1. Agonists may or may not be homologous to these natural compounds in
respect to conformation, charge or other characteristics. In any event, regardless of whether the
agonist is recognized in a manner similar to the "natural" ion channel, the agonist may cause
physiologic and/or biochemical changes within the cell, such that the cell reacts to the presence
of the agonist in the same manner as if the natural ion channel was present. Non-limiting
examples of agonists for TRPV3 ion channels are 2-aminoethoxydiphenyle borate (2-APB),
monoterpenes, including, but not limited to, those listed in Table A herein (e.g., camphor, (+)-
Borneol, (-)Isopinocampheol, (-)-Fenchone, (-)-Trans-pinocarveol, Isoborneol, (+)-
Camphorquinone, (-)-a-Thujone, alpha-pinene oxide, 1, 8-Cineole, (-)-alpha-Pinene, Isobornyl
acetate, 6-tert-butyl-m-cresol, Carvacrol, Dihydrocarbeol, Thymol, /3-xylenol, Kreosol,
Propofol, p-cymene, Carvacrol methylether, Dihydrocarveol, (-)-Carveol, and (-)-Isopulegol, (-)
methol, (-)-Carvone, (+)-Dihydrocarvone, (-)-Menthone, (+)-Limonene, Terpineol, (+)-Linalool
Geraniol, l-Isopropyl-4-methyl-bicyclo[3.1.0]hexan-4-ol, (-)-alpha-Bisabolol, mugetanol,
camphor oil, which contains other monoterpene constituents, including, but not limited to, those
listed in Table A herein.

[0039] As used herein "antagonist" refers to molecules or compounds which inhibit the action
of a "native" or "natural" molecule, such as TRPV3. Antagonists may or may not be
homologous to these natural compounds in respect to conformation, charge or other
characteristics. Thus, antagonists may be recognized as acting on the same receptor, in this case
TRPV3. Antagonists may have allosteric effects which prevent the action of an agonist (e.g., by
modifying a DNA adduct, or antagonists may prevent the function of the agonist (e.g., by
blocking a DNA repair molecule).

[0040] The terms "animal," "patient," or "subject" as used herein, mean any animal (e.g.,
mammals, (including, but not limited to humans, primates, dogs, cattle, cows, horses,
kangaroos, pigs, sheep, goats, cats, rabbits, rodents, and transgenic non-human animals), and
the like, which are to be the recipient of a particular treatment. Typically, the terms "animal" "subject" and "patient" are used interchangeably herein in reference to a human subject or a rodent. The preferred animal, patient, or subject is a human.

[0041] The term "2-aminooethoxydiphenyl borate" or "2-APB" as used herein, means a chemical that acts to inhibit IP3 receptors, Orai channels and TRP channels (although it activates TRPV1, TRPV2, & TRPV3 at higher concentrations) and derivatives thereof. In research it is used to manipulate intracellular release of calcium ions (Ca^{2+}) and modify TRP channel activity.

[0042] The term "2-aminooethoxydiphenyl borate" or "2-APB" derivative as used herein means any variation, deviation, change, or analog of the 2-APB molecule. This may include, but is not limited to a variation in stereochemistry to either increase or decrease the size of the ring, or such as an addition or deletion of a substituent, or a variation in functional group, or an analog. 2-APB derivatives or analogs are known in the art (e.g., Y, Dobrydneva et al., 2005).

[0043] The term "an individual at risk" as used herein, means one may or may not have detectable disease, and may or may not have displayed detectable disease prior to the treatment methods described herein. "At risk" denotes that an individual who is determined to be more likely to develop a symptom based on conventional risk assessment methods or has one or more risk factors that correlate with development of diabetes. An individual having one or more of these risk factors has a higher probability of developing SCC than an individual without these risk factors. Examples (i.e., categories) of risk groups are well known in the art and discussed herein.

[0044] The term "biomarker" as used herein, means any biological feature from an organism which is useful or potentially useful for measuring the initiation, progression, severity, pathology, aggressiveness, grade, activity, disability, mortality, morbidity, disease subclassification or other underlying feature of one or more biological processes, pathogenic processes, diseases, or responses to therapeutic intervention. For the present invention, the biomarkers of high risk SCC or high risk non-melanoma skin cancer are one or more of the proteins TRPV3, TRPV1, TRPA1, and TRPC1.

[0045] The term "camphor" as used herein, means a terpenoid with the chemical formula C_{10}H_{16}O. It is an organic compound of the isoprenoid family that belongs to the group of bicyclic monoterpenes. A white, waxy solid with a penetrating, somewhat musty aroma, it is
obtained from the wood of the camphor laurel (laurel family), *Cinnamomum camphora* (found in Asia), or produced synthetically from oil of turpentine. It exists in the optically active dextro and levo forms, and as the racemic mixture of the two forms. All of these melt within a degree of 178°C (352°F). The principal form is dextro-camphor, which occurs in the wood and leaves of the camphor tree (*Cinnamomum camphora*). Camphor is also synthesized commercially on a large scale from pinene which yields mainly the racemic variety. Camphor is readily absorbed through the skin and produces substance feeling of warmth, and acts as a slight local anesthetic and anti-itch substance. There are anti-itch gels and soothing gels with camphor as the active ingredient. Camphor is an active ingredient (along with menthol) in vapor-steam products, such as Vicks VapoRub. The IUPAC name for camphor is 1,7,7 Trimethylbicyclo[2.2.1]heptan-2-one. Since 1983, the Federal Food and Drug Administration (FDA) have banned the sale of products with more than 11% camphor because it can be toxic if ingested.

[0046] The term "camphor derivative" as used herein means any variation, deviation, or change in the camphor molecule. This may include, but is not limited to a variation in stereochemistry to either increase or decrease the size of the ring, or such as an addition or deletion of a substituent, or a variation in functional group, or an analog. The following are nonlimiting examples of camphor derivatives: 4-methyl-benzylidene camphor, [3-(4'-methyl)benzylidene-bornan-2-one] , 3-benzylidene camphor(3-benzylidene-bornan-2-one), polyacrylamidomethylbenzylidene camphor {N-[2(and 4)-2-oxyborn-3-ylidene-methyl]benzyl}acrylamide polymer}, trimonium-benzylidene camphor sulfate[3-(4'-trimethylammonium)-benzylidene-bornan-2-one methyl sulfate], terephthalydene dicamphorsulfonic acid {3,3'-(1,4-phenylenedimethine)-bis(7,7-dimethyl-2-oxo-bicyclo[2.2.1]heptane-1-methanesulfonic acid) or salts thereof, and benzylidene camphorsulfonic acid [3-(4'-sulfo)benzylidenebornan-2-one] or salts thereof. 4-Methylbenzylidene camphor. (4-MBC) is an organic camphor derivative that is used in the cosmetic industry for its ability to protect the skin against UV, specifically UV B radiation. As such it is used in sunscreen lotions and other skincare products claiming a SPF value. Its tradenames include Eusolex 6300 (Merck) and Parsol 5000 (DSM). Another example is Norcamphor which is a camphor derivative with the three methyl groups replaced by hydrogen. More specifically, Norcamphor is a chemical compound, classified as a ketone,
which is an analog of camphor without the three methyl groups. It is used as a chemical building block in organic synthesis.

[0047] The term, "camphor oil" as used herein means a colorless liquid obtained from the wood of the camphor tree (*Cinnamomum camphora*) by distillation and separation from the solid camphor, used in varnish, soaps, and shoe polish, and in medicine chiefly as a rubefacient. It is extracted from the wood by steam distillation. Chemical components include but are not limited to a-pinene, camphene, b-pinene, sabinene, phellandrene, limonene, 1,8-cineole, y-terpinene, p-cymene, terpinolene, furfural, camphor, linalool, bornyl acetate, terpinen-4-ol, caryophyllene, boraeol, piperitone, geraniol, safrole, cinnamic aldehyde, methyl cinnamate and eugenol. Camphor oil may be a natural extract or a synthetic mixture of components (*e.g.*, CAS number 8008-51-3). A natural extract or naturally derived camphor oil may have a different composition from lot to lot from that of a synthetic camphor oil. Monoterpenes other than camphor are major constituents of camphor oil. Several of these, including linalool, pinene, limonene, geraniol and borneol are TRPV3 agonists (see Vogt-Eisele et al Br J Pharmacol 2007 151:530-540; PMID 17420775) and include, but are not limited to those listed in Table A herein. In particular linalool has been reported to make up 90% of camphor oil in one study, and borneol is more effective than camphor as a TRPV3 agonist.

[0048] The term, "kit" as used herein, means any manufacture (*e.g.*, a package or container) comprising at least one reagent, *e.g.*, a TRPV3 agonist for treatment of SCC (including high risk forms), non-melanoma cancers (including high-risk forms), and AK. In certain embodiments, the manufacture may be promoted, distributed, or sold as a unit for performing the methods of the present invention.

[0049] The term "pain" as used herein, means an unpleasant sensation which may be associated with actual or potential tissue damage and which may have physical Nociception and emotional components. Nociception, on the other hand, is a neurophysiological term and denotes specific activity in nerve pathways.

[0050] The term "receptor" as used herein, means a structure expressed by cells and which recognizes binding molecules (*e.g.*, ligands).

[0051] The term "squamous cell carcinoma" ("SCC") as used herein, means a cancer of a kind of epithelial cell, the squamous cell that make up the main part of the epidermis of the
skin. SCC is one of the major forms of skin cancer. However, squamous cells also occur in the lining of the digestive tract, lungs, and other areas of the body, and SCC occurs as a form of cancer in diverse tissues, including the lips, mouth, esophagus, urinary bladder, prostate, lung, vagina, and cervix, among others. SCC is a histologically distinct form of cancer arising from the uncontrolled multiplication of cells of epithelium, or cells showing particular cytological or tissue architectural characteristics of squamous cell differentiation, such as the presence of keratin, tonofilament bundles, or desmosomes, structures involved in cell-to-cell adhesion. Squamous cell carcinomas are at least twice as frequent in men as in women. They rarely appear before age fifty and are most often seen in individuals in their seventies. The majority of skin cancers in African-Americans are squamous cell carcinomas, usually arising on the sites of preexisting inflammatory skin conditions or burn injuries. SCC is still sometimes referred to as "epidermoid carcinoma" and "squamous cell epithelioma," though the use of these terms has decreased.

The terms "high risk form of SCC" and "high risk form of non-melanoma cancer" as used herein mean SCC and non-melanoma cancers, respectively, that expresses either significantly higher or significantly lower than normal levels of mRNA encoding one or more of the proteins of TRPV3, TRPV1, TRPA1, and TRPC1, which are biomarkers of high risk SCC and non-melanoma cancers that permit one to make a definitive diagnosis. In the general literature prior to the discoveries described herein, a diagnosis of high-risk SCC was based entirely on a battery of clinical and histological criteria because there was no known biological marker. In general a surgeon refers to a high risk SCC as one that that has a greater risk for recurrence (following treatment) and metastasis, based on the following criteria:

Clinical features:

1. Size > 2cm
2. Anatomic site (particularly SCCs of lip and ear, other high risk sites include eyelids, nose, mucous membranes, scalp/forehead/temple, anogenital region)
3. Rapid growth
4. Tumors arising in injured or chronically diseased/inflamed skin
5. Immunosuppression
6. History of irradiation to skin
7. History of recurrence following previous treatment

Histologic features:
1. Tumor depth >4mm
2. Poorly differentiated histology
3. Perineural invasion

[0053] The term "monoterpenes" as used herein, are a group of naturally occurring organic compounds (like camphor, borneol or methol) derived from two isoprene units. Most of them are fragrant and form major constituents of many plant-derived essential oils. A number of monoterpenes have also been described as agonists or antagonists of different members of the transient receptor potential (TRP) channel family and are described herein in Table A.

[0054] The term "prophylactically effective amount" as used herein, means an amount of a drug that, when administered to a subject, will have the intended prophylactic effect, e.g., preventing or delaying the onset (or reoccurrence) of the disease or symptoms, or reducing the likelihood of the onset (or reoccurrence) of the disease or symptoms. The full prophylactic effect does not necessarily occur by administration of one dose and may occur only after administration of a series of doses. Thus, a prophylactically effective amount may be administered in one or more administrations. An "effective amount" of an agent is an amount that produces the desired effect.

[0055] The term "significantly higher" as used herein, means that levels of mRNA encoding the TRP channel (e.g., TRPV3, TRPV1, TRPA1, and TRPC1) in a subject biopsy are a statistically significant increase over control levels. On the other hand, the term "significantly lower" as used herein, means levels of mRNA encoding the TRP channel (e.g., TRPV3, TRPV1, TRPA1, and TRPC1) in a subject biopsy are a statistically significant decrease below control levels. Certain embodiments provide transcript levels that were determined to differ (either "significantly higher" or "significantly lower") between tissues by considering the variability of the qPCR assay. Means from qPCR technical replicates (typically 3-6 per sample) were compared with Student's t tests (two-tailed). Expression is therefore considered to be "significantly higher" or "significantly lower" if the t test indicates that the means differ at the P<0.05 level.

[0056] The term "therapeutically effective amount" as used herein means an amount that achieves the intended therapeutic effect of reducing or controlling or eliminating a keratinocyte-derived lesion such as SCC or AK, including precancerous lesions and benign tumors having the morphological characteristics of SCC in a subject. The full therapeutic effect does not
necessarily occur by administration of one dose and may occur only after administration of a
series of doses. Thus, a therapeutically effective amount may be administered in one or more
administrations per day for successive days.

[0057] The term "transient receptor potential vanilloid 3" or "TRPV3" protein as used herein,
means a nonselective calcium cation channel that is proposed to function in a variety of
processes, including temperature sensation and vasoregulation. The TRPV3 channel is widely
expressed in the human body, especially in the skin in keratinocytes, but also in the brain. It is a
thermosensitive ion channel expressed predominantly in the skin and neural tissues. It is
activated by warmth and the monoterpene camphor and has been hypothesized to be involved in
skin sensitization.

[0058] The term "treating" a disease such as SCC cancer (including high-risk forms), or non-
melanoma cancers (including high-risk forms), or AK in a patient as used herein, means taking
steps to obtain beneficial or desired results, including clinical results. For purposes of this
invention, beneficial or desired clinical results include, but are not limited to alleviation or
amelioration of one or more symptoms of the SCC cancer (including high-risk forms), or non-
melanoma cancers (including high-risk forms), or AK; diminishing the extent of disease;
delaying or slowing disease progression; amelioration and palliation or stabilization of the
disease state.

[0059] The terms "treat" or "treatment" as used herein, mean both therapeutic treatment and
prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an
undesired physiological change or disorder, such as the development, progression or spread of
cancer. For purposes of this invention, beneficial or desired clinical results include, but are not
limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not
worsening) state of disease, delay or slowing of disease progression, amelioration or palliation
of the disease state, and remission (whether partial or total), whether detectable or undetectable.
"Treatment" can also mean prolonging survival as compared to expected survival if not
receiving treatment. Those in need of treatment include those already having cancer and those
with benign tumors or precancerous lesions.

[0060] The term "cancer" as used herein, includes the enumerated diseases which are any
keratinocyte-derived lesions, e.g., a member of any class of SCC diseases, non-melanoma skin
cancers and actinic keratinosis (AK) characterized by the uncontrolled or aberrant growth of
aberrant keratinocytes. Cancer includes high-risk forms of SCC and high risk non-melanoma skin cancers and neoplastic conditions, whether characterized as malignant, benign, soft tissue, or solid, and cancers of all stages and grades including pre- and post-metastatic cancers. SCC cancers can appear on the skin, lips, mouth, lung, head, stomach, prostate, colon, rectum, throat, urinary tract, reproductive tract, and esophagus.

[0061] As used herein, a "tumor" comprises one or more cancer cells or benign cells or precancerous cells.

[0062] The term "sample" as used herein means, any biological specimen obtained from a subject. Samples include, without limitation, a tissue sample (e.g., tumor tissue) such as a biopsy of a tumor or of an area of skin having or suspected of having SCC (including high-risk forms), non-melanoma cancers (including high-risk forms), or AK, the tissue is typically exposed skin but can also be lips, mouth, esophagus, urinary bladder, prostate, lung, vagina, and cervix. A biopsy of cells of a solid tumor or of skin suspected of having SCC (including high-risk forms), non-melanoma cancers (including high-risk forms), or AK, can be obtained using any technique known in the art.

[0063] The term "transient receptor potential channel" or "TRP channel" as used herein, mean a group of ion channels located mostly on the plasma membrane of numerous human and animal cell types. There are about 28 TRP channels that share some structural similarity to each other. These are grouped into two broad groups: group 1 includes, TRPC ("C" for canonical), TRPV ("V" for vanilloid), TRPM ("M" for melastatin), TRPN and TRPA. In group 2, there are TRPP ("P" for polycystic) and TRPML ("ML" for mucolipin). Many of these channels mediate a variety of sensations like the sensations of pain, hotness, warmth or coldness, different kinds of tastes, pressure, and vision. In the body, some TRP channels are thought to behave like microscopic thermometers and used in animals to sense hot or cold. Some TRP channels are activated by molecules found in spices like garlic (allicin), chilli pepper (capsaicin), wasabi (allyl isothiocyanate); others are activated by menthol, camphor, peppermint, and cooling agents; yet others are activated by molecules found in cannabis (i.e. THC, CBD and CBN). Some act as sensors of osmotic pressure, volume, stretch, and vibration.

1. **Overview**

[0064] It has been discovered that agonists of TRPV3, a calcium-permeable cation channel, are
new avenues for the identification, diagnosis, and treatment of any of keratinocyte-derived cancerous or precancerous lesions such as any form of SCC, (including high risk SCC), non-melanoma skin cancers (including high risk non-melanoma skin cancers) and AK ("the enumerated diseases"). Certain embodiments are directed to diagnosing a high-risk form of SCC or high risk non-melanoma skin cancer by determining if the level of expression of mRNA encoding one or more TRP channels, e.g., TRPV3, TRPC1, TRPV1, TRPA1 in a biopsy of the lesion from a subject is either significantly higher or significantly lower compared to the corresponding level in normal tissue.

[0065] In another embodiment, the enumerated diseases are treated by applying one or more of the active agents, to the affected area for a period of time either before the area is surgically removed, and/or for a period of time after the surgery. In yet another embodiment, the invention is directed to pharmaceutical formulations and kits comprising them having one or more TRPV3 agonists, for example monoterpines (e.g., camphor), camphor oil, which contains other monoterpene constituents, and 2-APB. Other embodiments are directed to sunscreens that contain the active agents for the purposes of preventing keratinocyte-derived lesions from developing.

[0066] Non-melanoma skin cancers are the most common cancers in the United States. Little is known about the pathophysiological mechanisms underlying keratinocyte-derived skin cancers and their relation to normal keratinocyte growth and differentiation. The human epidermis is a multilayered stratified epithelium whose differentiation program is triggered in part by increased Ca^{2+} levels. The role of Ca^{2+} in epidermal physiology, including the molecular basis for differentiation, is not fully understood. It is known that Ca^{2+} triggers the commitment switch from keratinocyte proliferation to differentiation. As non-melanoma skin cancers typically exhibit perturbed patterns or dramatic loss of keratinocyte differentiation, it is important to determine the role for the calcium ion channels TRPV3, TRPV1, TRPA1, and TRPC1, in human keratinocyte maturation and its potential as a target for skin cancer therapy.

2. Background

[0067] The incidence of human squamous cell carcinomas and non-melanoma skin cancer is at an all-time high (Ratushny et al., 2012). Although the standard of care (e.g., surgery) is adequate for low risk cases, patients with more aggressive tumors show high incidence of recurrence. For example, SCCs that develop in chronic immunosuppression, such as in organ...
transplant recipients and patients with HIV, are frequently highly aggressive and potentially fatal tumors. Little is known about the pathophysiological mechanisms underlying keratinocyte-derived skin cancers and their relation to normal keratinocyte growth and differentiation. A better understanding of normal keratinocyte maturation is critical for unmasking the pathophysiological changes resulting in tumorigenesis. Environmental factors and genetic alterations that contribute to keratinocyte-derived skin cancers have been identified; however, a better understanding of normal keratinocyte physiology is critical for unmasking pathophysiological changes resulting in tumorigenesis.

[0068] In the healthy epidermis, calcium induces keratinocyte differentiation; therefore, calcium-permeable cation channels are posed to regulate this complex process. In vivo, the epidermis exhibits a calcium gradient necessary for normal structure and function: low calcium levels are found in the proliferative basal layer, whereas higher calcium levels induce keratinocyte differentiation in upper strata (Mascia et al, 2012). These conditions can be mimicked in vitro through a calcium-switch procedure. Normal keratinocytes proliferate in media containing 0.06 mM calcium and then are induced to differentiate by culturing them for a few days in >1 mM extracellular calcium. Calcium-triggered differentiation programs are dysregulated in non-melanoma skin cancers, leading to uncontrolled cellular proliferation.

[0069] Several calcium-permeable channels of the transient receptor potential (TRP) family have been implicated in normal keratinocyte function. Keratinocytes express TRPV3 and TRPV4 and mutations in these receptors cause epidermal abnormalities such as barrier defects and hyperkeratosis (Cheng et al, 2010; Chung et al, 2004b; Kida et al, 2012; Lin et al, 2012). In mice, TRPV3 regulates terminal keratinocyte differentiation via activation of EGFR signaling (Cheng et al, 2010). This tyrosine kinase pathway governs multiple steps in epidermal homeostasis, including keratinocyte proliferation and differentiation (Schneider et al, 2008). EGFR signaling also potentiates TRPV3 activity, illustrating that positive feedback links these receptors (Cheng et al, 2010). Several reports indicate that keratinocytes also express TRPV1, TRPA1 and TRPC1 (Denda & Tsutsumi, 2011). TRPV1 and TRPC1 are coupled to the epidermal growth factor receptor (EGFR) pathway by means of cytokine factor release and calcium signaling, respectively (Pan et al, 2011; Tajeddine & Gailly, 2012). Thus, in addition to being able to influence calcium influx, this link between TRP channels and the EGFR pathway indicates that these ion channels might regulate diseases associated with keratinocyte maturation.
EGFR overexpression is a characteristic feature of SCC, a keratinocyte-derived neoplasm that is the second most common malignancy in the US. Increased expression and activation of EGFR correlates with poor prognosis in SCC (Hardisson, 2003). A number of EGFR inhibitors are FDA-approved for SCC treatment but their efficacies are inconsistent and some are associated with severe skin toxicity (Bauman et al., 2012).

TRPV3

The movement of ions across cellular membranes is carried out by specialized proteins. TRP channels are one large family of non-selective cation channels that function to help regulate ion flux and membrane potential. These TRP channels are subdivided into six sub-families including the TRPV family. TRPV3 is a member of the TRPV class of TRP channels.

TRPV3 is a calcium permeable nonselective cation channel. In addition to calcium ions, TRPV3 channels are permeable to other cations, for example sodium. TRPV3 channels modulate membrane potential by modulating the flux of cations such as calcium and sodium ions. TRPV3 receptors are mechanistically distinct from voltage-gated calcium channels. Voltage-gated calcium channels respond to membrane depolarization and open to permit an influx of calcium from the extracellular medium that result in an increase in intracellular calcium levels or concentrations.

TRPV3 proteins are thermo sensitive channels expressed in skin cells (Peier et al. Science (2002), 296, 2046-2049) and dorsal root ganglion, trigeminal ganglion, spinal cord and brain (Xu et al. Nature (2002), 418, 181-185; Smith et al. Nature (2002), 418, 186-188). In keratinocyte cell lines, stimulation of TRPV3 leads to release of inflammatory mediators including interleukin-1. Therefore, TRPV3 may also play an important role in regulating inflammation and pain that results from the release of inflammatory stimuli. These mechanistic differences are accompanied by structural differences among voltage- gated and TRP channels.

Lingam, et al., Fused Pyrimidineone Compounds as TRPV3 modulators, 13/348,272 describes a method for treating a long list of diseases including AK by administering some newly discovered TRPV3 modulators, however all of the modulators he describes are TRPV3 antagonists as there is no mention of TRPV3 agonists. Furthermore, the compounds named have a different ring structure than either camphor or 2-APB. Moran et al., Compounds for Modulating TRPV3 Function, U.S. Apn. Serial No. 13/175366 similarly describes only TRPV3
antagonists and mentions agonists only generally in the context of screening assays. While Moran mentions that calcium, hence TRPV3, may play a role in SCC, it is not mentioned that TRPV3 should be stimulated with an agonist to treat SCC, nor indeed is any TRPV3 agonist identified; instead the entire focus is primarily on using TRPV3 antagonists therapeutically.

Squamous Cell Carcinoma

[0075] SCC is a cancer of a kind of epithelial cell, the squamous cell that makes up the main part of the epidermis of the skin. SCC is one of the major forms of skin cancer. However, squamous cells also occur in the lining of the digestive tract, lungs, and other areas of the body, and SCC occurs as a form of cancer in diverse tissues, including the lips, oral mucosa, nasopharynx, esophagus, urinary bladder, prostate, lung, vagina, and cervix, among others. SCC is a histologically distinct form of cancer arising from the uncontrolled multiplication of cells of epithelium, or cells showing particular cytological or tissue architectural characteristics of squamous cell differentiation, such as the presence of keratin, tonofilament bundles, or desmosomes, structures involved in cell-to-cell adhesion. Squamous cell carcinomas are at least twice as frequent in men as in women. They rarely appear before age 50 and are most often seen in individuals in their 70s. The majority of skin cancers in African-Americans are squamous cell carcinomas, usually arising on the sites of preexisting inflammatory skin conditions or burn injuries. SCC is still sometimes referred to as "epidermoid carcinoma" and "squamous cell epithelioma", though the use of these terms has decreased.

[0076] SCC is the second-most common cancer of the skin (after basal cell carcinoma) and it is more common than melanoma. World-wide, it is the most common cancer that has the potential to metastasize. It usually occurs in areas exposed to the sun. Sunlight exposure and immunosuppression are risk factors for SCC of the skin, with chronic sun exposure being the strongest environmental risk factor. Other risk factors include fair skin, age, male gender, history of skin cancer, and smoking. There is a risk of metastasis, often spreading to the lymph nodes, starting more than 10 years after diagnosable appearance of SCC. The risk of SCC metastasis is low, though it is much higher than with basal cell carcinoma. It is important to note that SCC of the lip and ears has significantly higher rates of local recurrence and distant metastasis (20-50%). SCC of the skin in individuals on immunotherapy or suffering from lymphoproliferative disorders (i.e. leukemia) also tend to be much more aggressive, regardless of their location. SCCs represent about 20% of the non-melanoma skin cancers, but due to their
more obvious nature and growth rates, they represent 90% of all head and neck cancers that are initially presented.

[0077] Squamous cell carcinoma is generally treated by surgical excision or Mohs surgery after biopsy. While it is relatively easy to identify SCC, it is more challenging to diagnose a high-risk form of SCC. Morphological factors that indicate a high-risk form of SCC include the depth of the tumor (at > 2cm), poorly differentiated cells, ulceration, the location of the area involved (e.g., ears, face, scalp are at higher risk), and intravascular invasion. Other high-risk factors for SCC include the immune status of the subject (immune compromised subjects have a higher risk of SCC), and whether or not the abnormal cells involve the nervous system. Patients receiving organ transplants ("OTR") are immune compromised and are at highest risk for metastatic SCCs.

[0078] High-risk forms of SCC and non-melanoma cancers are aggressive and therefore require more aggressive surgery (including taking wider margins, cutting deeper, and removing lymph nodes) as well as adjuvant therapy, than is needed if the SCC is not high risk. Until now, there were no biomarkers for distinguishing high-risk from non-high risk SCC and non-melanoma cancer. It has now been discovered that either a significantly higher or significantly lower level of mRNA encoding one or more of the following: TRPV3, TRPV1, TRPA1, and TRPC1 is a biomarker for high risk SCC and high risk non-melanomas. Therefore, SCC and non-melanoma biopsies can now be tested to determine whether they are high risk by determining if the expression of one or more of these biomarkers is significantly higher or significantly lower compared to a corresponding level in a normal subject. If the SCC or the non-melanoma is a high risk cancer, it warrants the most treatment: aggressive surgery and adjuvant therapy, including the newly described therapy of administering one or more TRPV3 agonists to treat the cancer. Even if the cancer is not high risk, treatment should include administration of therapeutically effective amounts of TRPV3 agonists as described herein, preferably before and after removal of the lesion and/or other treatment of the lesion. If the over or underexpression of the one or more of the biomarkers is detected in a biopsy from a subject, it is recommended that treatment, especially with the agonists, be started as soon as possible, even before the biopsy results are received. In the case of high risk SCC/high risk non-melanoma, treatment with TRPV3 agonists should be continued for a period of time after surgery to assure that all cancerous or precancerous cells are killed. It is preferred that all SCC
and non-melanoma subjects, both high risk and non-aggressive cancers, receive TRPV3 agonist therapy applied topically to the site from which the SCC was removed for an extended period of time, possibly indefinitely, to prevent abnormal keratinocyte proliferation and/or cancer recurrence.

Non-surgical options for the treatment of cutaneous SCC include topical chemotherapy, topical immune response modifiers, photodynamic therapy (PDT), radiotherapy, and systemic chemotherapy. Radiation therapy is a primary treatment option for patients in whom surgery is not feasible and is an adjuvant therapy for those with metastatic or high-risk cutaneous SCC. At this time, systemic chemotherapy is used exclusively for patients with metastatic disease. Mohs surgery, also known as chemosurgery, enables the surgeon to obtain complete margin control during removal of a skin and it allows for the removal of a skin cancer with very narrow surgical margin and a high cure rate.

**Actinic keratosis**

Actinic keratosis (also called "solar keratosis" and "senile keratosis," herein "AK") is a premalignant condition of thick, scaly, or crusty patches of skin. It is more common in fair-skinned people and it is associated with those who are frequently exposed to the sun, as it is usually accompanied by solar damage. AKs are pre-cancerous lesions, as some progress to squamous cell carcinoma, so treatment is recommended. Untreated lesions have up to 20% risk of progression to squamous cell carcinoma. People who take immunosuppressive drugs, such as organ transplant patients, are 250 times more likely to develop AK that may lead to skin cancer.

Medicated creams and solutions are typically used topically to treat actinic keratosis. The use of topical therapy, such as Imiquimod cream and PDT is generally limited to premalignant (i.e., AKs) and in situ lesions. 5-fluorouracil (5-FU) ointment or liquid in concentrations from 0.5 to 5 percent has FDA approval and is the most widely used topical treatment for AK as it is effective against not only the surface lesions but also the subclinical ones. Rubbed gently onto the lesions once or twice a day for two to four weeks, it produces cure rates of up to 93 percent. Imiquimod 5% cream, also FDA-approved, works by stimulating the immune system to produce interferon, a chemical that destroys cancerous and precancerous cells. It is rubbed gently on the lesion twice a week for four to sixteen weeks. Diclofenac is a non-steroidal anti-inflammatory drug used in combination with hyaluronic
acid. The resulting gel is applied twice a day for two to three months to prevent an inflammatory response, so this topical is well-tolerated. The hyaluronic acid delays uptake of the diclofenac, leading to higher concentrations in the skin. The gel, used in 0.015% or 0.05% concentrations depending on the AK site, is the first topical therapy to effectively treat AKs in just two or three days. Certain embodiments are directed to pharmaceutical formulations for topical applications of the above listed formulations that further include one or more of the active agents as described herein. Cryosurgery is the most commonly used treatment method when a limited number of lesions exist. Other treatment includes laser surgery, and photodynamic therapy.

3. Summary of Experimental Results and Embodiments of the Invention

[0082] In summary, it has been discovered that agonists of TRPV3, have therapeutic use in treating any cancerous or precancerous keratinocyte-derived lesions including SCC (including high-risk forms), non-melanoma skin cancers (including high-risk forms), and AK. The function of TRPV3 was examined in the maturation of normal human epidermal keratinocytes. Human biopsy-derived SCC cells were cultured in vitro in a preclinical organotypic model, found to have functional TRPV3, and responded to the TRPV3 agonist, 2-APB, by reducing cancer cell proliferation thereby reducing tumor burden and invasion. Topical camphor-oil treatment in vivo also reduced tumor burden and invasion, promoted regression of pre-malignant skin tumors and slowed progression of benign tumors to SCC. Dose-dependent effects of 2-APB and camphor were also observed on normal keratinocyte behavior. The following is a summary of results of experiments described in the Examples of this application.

- Identification of epidermal TRP channels as key regulators of the commitment switch from proliferation to differentiation in human epidermal keratinocytes;
- TRP-channel agonists induce fate switch from proliferation to differentiation in human keratinocytes;
- Constitutive treatment with 2-APB or camphor arrests human keratinocyte proliferation and promotes differentiation;
- TRP-channel gene expression and protein function is upregulated in normal human epidermal keratinocytes upon differentiation;
- TRP-channel gene expression (TRPV3, TRPV 1, tRPA 1, and TRPC 1) is dysregulated
in human high-risk SCC biopsies compared with normal skin;

- Quantitative expression analysis demonstrated that TRPV1, TRPV3, TRPC1 transcript levels were higher in keratinocytes cultured in differentiation compared with proliferative conditions;

- Calcium imaging revealed that the responses of normal human epidermal keratinocytes to acute application of camphor or 2-APB increased upon differentiation;

- 24-hour incubation with 2-APB in low-calcium growth conditions boosted the expression of differentiation genes;

- 50 μM 2-APB or 4-8 mM camphor caused cell-cycle arrest, a hallmark for the commitment switch from proliferation to differentiation;

- Stimulating TRP-channel activity is sufficient to induce early differentiation which enhanced TRP-mediated calcium signaling, caused either by raising extracellular calcium or by exposure to TRP agonists, promotes differentiation in normal human epidermal keratinocytes;

- 2-APB reduced human SCC tumor growth and dermal invasion in vitro;

- Topical camphor-oil treatment promotes regression of pre-malignant skin tumors in vivo;

- Topical camphor-oil treatment slows progression of benign tumors to SCC in vivo;

- Topical camphor-oil treatment suppresses SCC proliferation and invasion in vivo;

- Topical camphor-oil treatment dramatically attenuates malignant SCC conversion in vivo;

- Topical camphor-oil treatment is sufficient to completely clear tumors in a subset of animals in carcinogenesis models in vivo; and

- Treatment with camphor upregulates levels of a keratinocyte terminal differentiation marker in vivo.

[0083] In the present specification, the invention has been described with reference to specific embodiments thereof. It will, however, be evident that various modifications and changes may be made thereto without departing from the broader spirit and scope of the invention. The
specification and drawings are, accordingly, to be regarded in an illustrative rather than a restrictive sense. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference as if set forth herein in their entirety, except where terminology is not consistent with the definitions herein. Although specific terms are employed, they are used as in the art unless otherwise indicated.

4. Pharmaceutical Compositions or Formulations and Their Administration

[0084] Embodiments are directed to pharmaceutical compositions that contain one or more TRPV3 agonists or derivatives thereof (e.g., monoterpenes listed in Table A, preferably camphor, camphor oil which contains other monoterpene constituents, and 2-APB) for treatment or prevention of keratinocyte-derived lesions, e.g., SCC (including high-risk forms), non-melanoma skin cancers (including high-risk forms), and AK. Other embodiments are directed to sunscreens containing one or more of these TRPV3 agonists. These pharmaceutical compositions and kits comprising them may be formulated as described below and are typically in topical formulations including creams, ointments, paste, powers, lotions, and gels, preferably in an ointment that is topically applied and is hydrophobic enough to keep the camphor in the ointment but also hydrophilic enough that it would not dry the skin and would be tolerated daily. Preferably, the contemplated pharmaceutical compositions include the active agents described herein in an amount sufficient to treat one or more of the enumerated diseases: SCC, (including high-risk forms) non-melanoma skin cancers (including high risk forms), or AK in a subject or prevent reoccurrence. The doses of active agents for formulations to treat the enumerated diseases are discussed below.

A. Pharmaceutical Compositions or Formulations Containing Camphor or 2-APB.

[0085] In a preferred embodiment, the pharmaceutical composition for treatment of a keratinocyte-derived cancerous or precancerous lesion (SCC (including high-risk forms) non-melanoma cancer (including high-risk forms), or AK) is comprised of one or more TRPV3 agonists such as camphor and, 2-APB. The following are commercial camphor products known in the art:

Balmosa Cream (camphor 4%, menthol 2%, methyl salicylate 4%, capsicum oleoresin 0.035%) (Pharmax Healthcare)
Boots Vapour Rub (Boots)
Earex (almond oil 33.33%, arachis oil 33.33%, camphor oil 33.33%) Seton Healthcare
Mentholatum Vapour Rub (camphor 9%, menthol 1.35%, methyl salicylate 0.33%) (Mentholatum)
Nasciodine (iodine 1.26%, menthol 0.59%, methyl salicylate 3.87%, turpentine oil 3.87%, camphor 3.87%)
Nicobrevin (methyl valerate 100mg, quinine 15mg, camphor 10mg, eucalyptus oil 10mg) (Intercare Products)
PR Heat Spray (camphor 0.62%, methyl salicylate 1.24%, ethyl nicotinate 1.1%) (Crookes Healthcare)
Radian-B (liniment and spray: menthol 1.4%, camphor 0.6%, ammonium salicylate 1%, salicylic acid 0.54%. rub: menthol 2.54%, camphor 1.43%, methyl salicylate 0.42%, capsicin 0.042%. cream: camphor 1.43%, menthol 2.54%, methyl salicylate 0.42%, oleoresin capsicum 0.005%) Roche Consumer Health
Tixylix inhalant (camphor 60mg, menthol 25mg, turpentine oil 50mg, eucalyptus oil 20mg) (Intercare Products)
Vicks Inhaler (camphor 41.54%, menthol 41.54%, Siberian pine needle oil 4.65%) %) (Procter and Gamble)
Vicks Sinex (oxymetazoline 0.05%, menthol 0.025%, camphor 0.015%, eucalyptus oil 0.0075%) %) (Procter and Gamble) and
Vicks Vaporub (menthol 2.82%, camphor 5.46%, eucalyptus oil 1.35%, turpentine oil 4.71%) (Procter and Gamble).

[0086] The following are non-proprietary preparations known in the art:

Camphor Linctus compound (APF): Camphor spirit compound 1ml, glycerol 1.5ml, tolu syrup to 5ml.
Camphor Liniment (BP 1973): Camphor 20% w/w in arachis oil (AKA Camph. Lin; Camphorated oil).
Camphor Spirit (USP): Camphor 10g, alcohol to 100ml.
Concentrated Camphor Water (BP) Camphor 4g, alcohol (90%) 60ml, water to 100ml.

[0087] In other embodiments one or more active agents, preferably camphor or 2-APB in sunscreens, (also commonly known as sun block, sun tan lotion, sun screen, sunburn cream or block out) formulated for topical application as a lotion, spray, gel or other product that absorbs or reflects some of the sun's ultraviolet (UV) radiation on skin exposed to sunlight and thus helps protect against sunburn. Depending on the mode of action, sunscreens can be classified into physical sunscreens (i.e., those that reflect the sunlight) or chemical sunscreens (i.e., those that absorb the UV light). Sunscreens contain one or more of the following ingredients: (i) organic chemical compounds that absorb ultraviolet light; (ii) inorganic particulates that reflect, scatter, and absorb UV light (such as titanium dioxide, zinc oxide, or a combination of both); and (iii)
organic particulates that mostly absorb light like organic chemical compounds, but contain multiple chromophores, may reflect and scatter a fraction of light like inorganic particulates, and behave differently in formulations than organic chemical compounds.

[0086] A wide variety of sun screening agents are described in U.S. Pat. No. 5,087,445, to Haffey et al. U.S. Pat. No. 5,073,372, to Turner et al., U.S. Pat. No. 5,073,371, to Turner et al. and Segarin, et al., at Chapter VIII, pages 189 et seq., of Cosmetics Science and Technology all of which are incorporated herein by reference in their entirety. Preferred among those sunscreens which are useful in the composition of the instant invention are those selected from the group consisting of 2-ethylhexyl p-methoxycinnamate, octyl methoxycinnamate, 1-p-aminobenzoate, p-aminobenzoic acid, 2-phenylbenzimidazole- 5-sulfonic acid, octocrylene, oxybenzone, homomenthyl salicylate, octyl salicylate, 4,4’-methoxy-t-butylidibenzoylmethane, 4-isopropyl dibenzoylmethane, 3-benzylidene camphor, 3-(4-methylbenzylidene) camphor, titanium dioxide, zinc oxide, silica, iron oxide, and mixtures thereof. Still other useful sunscreens are those disclosed in U.S. Pat. No. 4,937,370, to Sabatelli and U.S. Pat. No. 4,999,186, to Sabatelli et al. The sun screening agents disclosed herein have, in a single molecule, two distinct chromophore moieties which exhibit different ultra-violet radiation absorption spectra. One of the chromophore moieties absorbs predominantly in the UVB radiation range and the other absorbs strongly in the UVA radiation range. These sun screening agents provide higher efficacy, broader UV absorption, lower skin penetration and longer lasting efficacy relative to conventional sunscreens. Especially preferred examples of these sunscreens include those selected from the group consisting of 4-N,N-(2-ethylhexyl)methylanminobenzoic acid ester of 2,4- hydroxybenzophenone, 4-N,N-(2-ethylhexyl)methylanminobenzoic acid ester with 4- hydroxydibenzoylmethane, 4-N,N-(2-ethylhexyl)methylanminobenzoic acid ester of 2-hydroxy-4-(2-hydroxyethoxy)benzophenone, 4-N,N-(2-ethylhexyl)-methylaminobenzoic acid ester of 4-(2-hydroxyethoxy)dibenzoylmethane, and mixtures thereof. Generally, the sunscreens can comprise from about 0.5 percent to about 20 percent of the compositions useful herein. Exact amounts will vary depending upon the sunscreen formulation chosen, the particular active agent, and the desired Sun Protection Factor (SPF). SPF is a commonly used measure of photoprotection of a sunscreen against erythema. See Federal Register, Vol. 43, No. 166, pp. 38206-38269, Aug. 25, 1978.

[0088] Medical organizations such as the American Cancer Society recommend the use of
sunscreen because it aids in the prevention of developing squamous cell carcinomas and basal-cell carcinomas. However, the use of sunscreens is controversial for various reasons. Many sunscreens do not block UVA radiation, which does not cause sunburn but can increase the rate of melanoma, another kind of skin cancer, and photodermatitis, so people using sunscreens may be exposed to high UVA levels without realizing it. The use of broad-spectrum (UVA/UVB) sunscreens can address this concern.

[0089] The following are the FDA allowable active ingredients in sunscreens that can be used in sunscreen formulations comprising the active agents:

<table>
<thead>
<tr>
<th>UV-filter</th>
<th>Other names</th>
<th>Maximum concentration</th>
<th>Permitted in these countries</th>
<th>Results of safety testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Aminobenzoic acid</td>
<td>PABA</td>
<td>15% (EC-banned from sale to consumers from 8 October 2009)</td>
<td>USA, AUS</td>
<td>Protects against skin tumors in mice. Shown to increase DNA defects, however, and is now less commonly used.</td>
</tr>
<tr>
<td>Padimate O</td>
<td>OD-PABA, octyldimethyl-PABA, σ-PABA</td>
<td>8% (EC, USA, AUS) 10% (JP) (Not currently supported in EU and may be delisted)</td>
<td>EC, USA, AUS, JP</td>
<td>Not tested</td>
</tr>
<tr>
<td>Phenylbenzimidazole sulfoxonic acid</td>
<td>Ensulizole, Eusolex 232, PBSA, Parsol HS</td>
<td>4% (US, AUS) 8% (EC) 3% (JP)</td>
<td>EC USA, AUS, JP</td>
<td>Genotoxic in bacteria</td>
</tr>
<tr>
<td>Cinoxate</td>
<td>2-Ethoxyethyl p-methoxycinnamate</td>
<td>3% (US) 6% (AUS)</td>
<td>USA, AUS</td>
<td>Not tested</td>
</tr>
<tr>
<td>Dioxybenzene</td>
<td>Benzophenone-8</td>
<td>3%</td>
<td>USA, AUS</td>
<td>Not tested</td>
</tr>
<tr>
<td>Ingredient</td>
<td>Description</td>
<td>Concentration</td>
<td>Country</td>
<td>Toxicity</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>---------------</td>
<td>---------</td>
<td>---------------</td>
</tr>
<tr>
<td>Oxybenzone</td>
<td>Benzophenone-3, Escalol 567</td>
<td>6% (US) 10% (AUS,EU) 5% (JP)</td>
<td>EC, USA, AUS, JP</td>
<td>Not tested</td>
</tr>
<tr>
<td>Homosalate</td>
<td>Homomethyl salicylate, Escalol 557</td>
<td>10% (EC, JP) 15% (US,AUS)</td>
<td>EC, USA, AUS, JP</td>
<td>Not tested</td>
</tr>
<tr>
<td>Menthol anthranilate</td>
<td>Meradimate</td>
<td>5%</td>
<td>USA, AUS</td>
<td>Not tested</td>
</tr>
<tr>
<td>Octocrylene</td>
<td>Eusolex OCR, 2-Cyano-3,3-diphenyl</td>
<td>10%</td>
<td>ECUSA, AUS, JP</td>
<td>Increases ROS</td>
</tr>
<tr>
<td>Octyl methoxycinnamate</td>
<td>Octinoxate, EMC, OMC, Ethylhexyl methoxycinnamate, Escalol 557, Escalol 577, Parsol MCX</td>
<td>7.5% (US) 10% (EC,AUS)20% (JP)</td>
<td>ECUSA, AUS, JP</td>
<td></td>
</tr>
<tr>
<td>Octyl salicylate</td>
<td>Octisalate, 2-Ethylhexyl salicylate, Escalol 587, Escalol 577</td>
<td>5% (EC,USA,AUS) 10% (JP)</td>
<td>ECUSA, AUS, JP</td>
<td>Not tested</td>
</tr>
<tr>
<td>Sulisobenzone</td>
<td>2-Hydroxy-4-Methoxybenzophenone - 5-sulfonic acid, 3-Benzooyl-4-hydroxy-6-methoxybenzenesulfonic acid, Benzophenone-4, Escalol 577</td>
<td>5% (EC) 10% (US, AUS, JP)</td>
<td>ECUSA, AUS, JP</td>
<td></td>
</tr>
<tr>
<td>Trolamine salicylate</td>
<td>Triethanolamine salicylate</td>
<td>12%</td>
<td>USA, AUS</td>
<td>Not tested</td>
</tr>
<tr>
<td>Avobenzone</td>
<td>1-(4-methoxyphenyl)-3-(4-tert-butyl phenyl)propane-1,3-dione, Butyl methoxy dibenzoylethane, BMDBM, Parsol 1789,</td>
<td>3% (US) 5% (EC,AUS)10% (JP)</td>
<td>EC, USA, AUS, JP</td>
<td>Not Available</td>
</tr>
<tr>
<td>Ecamsule</td>
<td>Mexoryl SX, Terephthalylidene Dicamphor Dicamphor Sulfonic Acid</td>
<td>10%</td>
<td>EC,AUS</td>
<td>Protects against skin tumors in mice</td>
</tr>
</tbody>
</table>

acrylic acid, 2-ethylhexyleste
Titanium dioxide  CI77891  25% (No limit Japan)  ECUSA, AUS, JP  Not tested

Zinc oxide  25% (US) 20% (AUS) (EC-25% provided particle size >100 nm)  ECUSA, AUS, JP  Protects against skin tumors in mice (Japan, No Limit)

B. Pharmaceutical Compositions or Formulations Containing Camphor Derivatives

[0090] Certain embodiments are directed to pharmaceutical compositions or formulations containing derivatives of camphor. The term "camphor derivative" as used herein means any variation, deviation, or change in the camphor molecule. This may include, but is not limited to a variation in stereochemistry to either increase or decrease the size of the ring, or such as an addition or deletion of a substituent, or a variation in functional group, or an analog. The following include, but are not limited to examples of camphor derivatives: 4-methylbenzylidene camphor, [3-(4'-methyl)benzylidene-bornan-2-one], 3-benzylidene camphor(3-benzylidene-bornan-2-one), polyacrylamidomethylbenzylidene camphor {N-[2(and 4)-2-oxysol-3-ylidene-methyl]benzyl}acrylamide polymer}, trimonium-benzylidene camphor sulfate[3-(4'-trimethylammonium)-benzylidene-bornan-2-one methyl sulfate], terephthalidene dicamphorsulfonic acid {3,3'-(1,4-phenylenedimethine)-bis(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hepta-ne-1-methanesulfonic acid) or salts thereof, and benzylidene camphorsulfonic acid [3-(4'-sulfo)benzylidenebornan-2-one] or salts thereof. 4-Methylbenzylidene camphor (4-MBC) is an organic camphor derivative that is used in the cosmetic industry for its ability to protect the skin against UV, specifically UV B radiation. As such it is used in sunscreen lotions and other skincare products claiming a SPF value. Its tradenames include Eusolex 6300 (Merck) and Parsol 5000 (DSM).

[0091] Other derivatives may include, e.g., salts of 10-camphorsulphonic acid (CSA) that are used in pharmaceutical preparations as an aqueous soluble form of camphor. Camphorsulfonic acid is a white crystalline acid C_{10}H_{15}OSO_{3} made by reaction of camphor with sulfuric acid and acetic anhydride. The most frequently found are camphorsulphonates of
sodium, codein, piperazine, ephedrine, and ethylmorphine, which are used in tablets, suppositories, oral drops, syrups and injections. The raw material for the preparation of camphorsulphonates for medical use is synthetic camphor, a mixture of optical isomers.

[0092] Other camphor derivatives of the invention may include a structural change in the molecule, such as norcamphor. Norcamphor has three methyl groups replaced by hydrogen. Therefore, embodiments of the present invention may include camphor derivatives that may or may not carry a secondary hydroxyl group on the ring.

[0093] Structural variations, derivatives, or changes in molecules are known in the art. For example, camphor belongs to the group of the bicyclic monoterpenes, other monoterpenes belonging to the monocyclic group that are structurally similar are also highly effective TRPV3 agonists. It is known in the art that other monoterpenes similar in structure to camphor carry a secondary hydroxyl group. Oxidation to a carbonyl group reduced the activity of the substance drastically, arguing that a hydroxyl group is a structural requirement for efficient activation of TRPV3. Structurally, the position of the hydroxyl group on the ring does not appear to be critical for TRPV3 activation in aromatic substances, but it is relevant for non-aromatic compounds such as dihydrocarveol and (-)-carveol (where the hydroxyl group in the meta position to the isopropyl residue), rather than in the ortho positions as in (-)-isopulegol and (-)-menthol.

C. Pharmaceutical Compositions or Formulations Containing Camphor-Oil Which Contain Monoterpene Constituents

[0094] Certain embodiments are directed to pharmaceutical compositions that contain camphor-oil which contains (in addition to camphor oil) other monoterpene constituents (as shown in Table A herein). Camphor-oil is a colorless liquid obtained from the wood of the camphor tree (Cinnamomum camphora) by distillation and separation from the solid camphor, used in varnish, soaps, and shoe polish, and in medicine chiefly as a rubefacient. It is extracted from the wood by steam distillation. Chemical components of camphor oil besides camphor include but are not limited to a-pinene, camphene, b-pinene, sabinene, phellandrene, limonene, 1,8-cineole, y-cinuprene, p-cymene, cernoiene, furfural, camphor, linaiooi, bornyl acetate, terpinen-4-oi, caryophyliene, borneol, piperitone, geranioi, safrole, cinnamaldehyde, methyl cinnamate and eugenol. Camphor oil can be a natural extract or a synthetic mixture of components (e.g., CAS number 8008-51-3). Monoterpenes (like camphor, borneol or methol) comprise a group of naturally occurring organic compounds driven from two isoprene units. Most of them are
fragrant and form major constituents of many plant-derived essential oils. While most commonly used as antimicrobial agents, monoterpenes have a wide range of applications in pharmaceutical, medical and cosmetic fields. These uses range from anesthetic and analgesic (Galeotti et al., 2001, 2002; Xu et al., 2005) to anti-inflammatory (Santos and Rao, 2001) and antipruritic applications (Umezu et al., 2001; Anand, 2003). Different monoterpenes have been shown to activate, inactivate or modulate ion channels. A number of monoterpenes have also been described as agonists or antagonists of different members of the transient receptor potential (TRP) channel family (Mckemy et al., 2002; Peier et al., 2002a, b; Behrendt et al., 2004; Moqrich et al., 2005; Xu et al., 2005, 2006; Macpherson et al., 2006). In addition to camphor, carvacrol, thymol and menthol have been shown to activate TRPV3. AK Vogt-Eisele et al., 2007 screened the activity of 33 monoterpenes on TRPV3. See Table A below.

Table A. Monoterpenoid agonists of TRPV3 AK Vogt-Eisele et al.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camphor</td>
<td>(+)-Borneol</td>
</tr>
<tr>
<td>(-)-Isopinocampeol</td>
<td>(-)-Fenchone</td>
</tr>
<tr>
<td>(-)-Trans-pinocarveol</td>
<td>Isoborneol</td>
</tr>
<tr>
<td>(+)-Camphorquinone</td>
<td>(-)-alpha-Thujone</td>
</tr>
<tr>
<td>Alpha-pinene oxide</td>
<td>1,8-Cineole</td>
</tr>
<tr>
<td>(-)-alpha-Pinene</td>
<td>Isobornyl acetate</td>
</tr>
<tr>
<td>6-tert-butyl-m-cresol</td>
<td>Carvacrol</td>
</tr>
<tr>
<td>Thymol</td>
<td>p-xylenol</td>
</tr>
<tr>
<td>Kreosol</td>
<td>Propofol</td>
</tr>
<tr>
<td>p-cymene</td>
<td>Carvacrol methylether</td>
</tr>
<tr>
<td>Dihydrocarveol</td>
<td>(-)-Carveol</td>
</tr>
<tr>
<td>(-)-Isopulegol</td>
<td>(-)-Menthol</td>
</tr>
<tr>
<td>(-)-Carvone</td>
<td>(+)-Dihydrocarvone</td>
</tr>
<tr>
<td>(-)-Menthone</td>
<td>(+)-Limonene</td>
</tr>
<tr>
<td>Terpineol</td>
<td>(+)-Linalool</td>
</tr>
<tr>
<td>Geraniol</td>
<td>1-Isopropyl-4-methyl-bicyclo [3.1.0] hexan-4-ol</td>
</tr>
<tr>
<td>(-)alpha-Bisabolol</td>
<td>Mugetanol</td>
</tr>
</tbody>
</table>
Monoterpenes other than camphor are major constituents of camphor oil. In certain embodiments, pharmaceutical compositions containing camphor oil may also include monoterpenes described above in Table A. More preferably, the monoterpene constituents may include linalool, pinene, limonene, geraniol, and borneol, but may be one of those listed in Table A or known in the art. In particular, linalool has been reported to make up 90% of camphor oil, and the bicyclic compound (+)-borneol is more effective than camphor as a TRPV3 agonist.

D. Form of and Delivery of Pharmaceutical Compositions or Formulations Containing Camphor or 2-APB, Derivatives thereof, and Camphor-Oil Which Contains Monoterpene Constituents

The pharmaceutical compositions or formulations comprising the active agents may exist in a wide variety of presentation forms, for example: in the form of liquid preparations as emulsions known in the art, or microemulsions gels, oils, creams, milk or lotions, powders lacquers, tablets or make-up, a stick, sprays (with propellant gas or pump-action spray) or aerosols, foams, or pastes.

Embodiments are also directed to cosmetic preparations for the skin comprising one or more active agents including light-protective preparations, such as sun milks, lotions, creams, oils, sunblocks or tropicals, pretanning preparations or after-sun preparations, also skin-tanning preparations, for example self-tanning creams. Of particular interest are sun protection creams, sun protection lotions, sun protection milk and sun protection preparations in the form of a spray.

Topical formulations of the active agents are preferred. Delivery may occur via dropper or applicator stick, as a mist via an aerosol applicator, via an intradermal or transdermal patch, or by simply spreading a formulation of the invention onto the affected area with fingers. Camphor is well absorbed after inhalation, (if formulated at a dose that is not toxic systemically) or dermal exposure (Baselt and Cravey 1990).

Pharmaceutical compositions of the invention may also include one or more emollients. An emollient is an oleaginous or oily substance, which helps to smooth and soften the skin, and may also reduce its roughness, flaking, cracking or irritation. Typical suitable emollients include mineral oil having a viscosity in the range of 50 to 500 centipoise (cps), lanolin oil, coconut oil, cocoa butter, olive oil, almond oil, macadamia nut oil, aloe extracts such as aloe vera lipoquinone, synthetic jojoba oils, natural sonora jojoba oils, safflower oil, corn oil, liquid lanolin, cottonseed oil and peanut oil. In some embodiments, the emollient is a cocoglyceride,
which is a mixture of mono, di and triglycerides of cocoa oil, sold under the trade name of Myritol 331 from Henkel KGaA, or Dicapryl Ether available under the trade name Cetiol OE from Henkel KGaA or a C_{12}-C_{15} Alkyl Benzoate sold under the trade name FinsolvTN from Finetex. Another suitable emollient is DC 200 Fluid 350, a silicone fluid.

[0100] Other suitable emollients include squalane, castor oil, polybutene, sweet almond oil, avocado oil, calophyllum oil, ricin oil, vitamin E acetate, olive oil, silicone oils such as dimethylopolysiloxane and cyclomethicone, linolenic alcohol, oleyl alcohol, the oil of cereal germs such as the oil of wheat germ, isopropyl palmitate, octyl palmitate, isopropyl myristate, hexadecyl stearate, butyl stearate, decyl oleate, acetyl glycerides, the octanoates and benzoates of (C^{n}-Cis) alcohols, the octanoates and decanoates of alcohols and polyalcohols such as those of glycol and glycercyl, ricinoleates esters such as isopropyl adipate, hexyl laurate and octyl dodecanoate, dicaprylyl maleate, hydrogenated vegetable oil, phenyltrimethicone, jojoba oil and aloe vera extract.

[0101] Still other suitable emollients which are solids or semi-solids at ambient temperatures may be used. Such solid or semi-solid cosmetic emollients include glyceryl dilaurate, hydrogenated lanolin, hydroxylated lanolin, acetylated lanolin, petrolatum, isopropyl lanolate, butyl myristate, cetyl myristate, myristyl myristate, myristyl lactate, cetyl alcohol, isostearyl alcohol and isocetyl lanolate. One or more emollients can optionally be included in the present invention ranging in amounts from about 1 percent to about 10 percent by weight, preferably about 5 percent by weight.

[0102] Certain embodiments may be in the form of a topical formulations that include an emulsion. These topical formulations can be in the form of the following:

- **Cream -** Emulsion of oil and water in approximately equal proportions. Penetrates stratum corneum outer layer of skin well.

- **Ointment -** Combines oil (80%) and water (20%). Effective barrier against moisture loss.

- **Gel -** Liquefies upon contact with the skin.

- **Paste -** Combines three agents - oil, water, and powder; an ointment in which a powder is suspended.
Powder - A finely subdivided solid substance

Topical carriers for use in embodiments of the invention are disclosed in REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY 282-291 (Alfonso R. Gennaro ed. 19th ed. 1995). Suitable gels for use in the invention are disclosed in REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY 1517-1518 (Alfonso R. Gennaro ed. 19th ed. 1995), U.S. Pat. No. 6,387,383 (issued May 14, 2002); U.S. Pat. No. 6,517,847 (issued Feb. 11, 2003); and U.S. Pat. No. 6,468,989 (issued Oct. 22, 2002). Dow Coming Corp. As used herein, a pharmaceutically acceptable topical carrier is any pharmaceutically acceptable formulation that can be applied to the skin surface for topical, dermal, intradermal, or transdermal delivery of a pharmaceutical or medicament. Pharmaceutical compositions of the invention are typically prepared by mixing a TRPV3 agonist, (e.g., camphor, camphor oil, which contains other monoterpenic constituents, and 2-APB or derivatives thereof) with a topical carrier according to well-known methods in the art. The topical carriers include pharmaceutically acceptable solvents, such as a polyalcohol or water; emulsions (either oil-in-water or water-in-oil emulsions), such as creams or lotions; micro emulsions; gels; ointments. Suitable protectives and adsorbents include, but are not limited to, dusting powders, zinc stearate, collodion, dimethicone, silicones, zinc carbonate, aloe vera gel and other aloe products, vitamin E oil, allatoin, glycerin, petrolatum, and zinc oxide.

Topical pharmaceutical compositions of the invention in the form of an emulsion may optionally contain drying agents. Drying agents generally promote rapid drying of moist areas and coats the skin for protection and healing. In particular, it acts to prevent irritation of the involved area and water loss from the skin layer by forming a physical barrier on the skin. Preferred drying agents include calamine; zinc containing drying agents such as zinc oxide, zinc acetate, zinc stearate, zinc sulfate, copper sulfate, kaolin, potassium permanganate, Burow's aluminum solution, talc, starches such as wheat and corn starch, silver nitrate, and acetic acid.

Or these pharmaceutical compositions or formulations may be in the form of an aqueous solution or suspension, preferably, an aqueous solution. Suitable aqueous topical formulations for use in the invention are disclosed in REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY 1563-1576 (Alfonso R. Gennaro ed. 19th ed. 1995). Other suitable aqueous topical carrier systems are disclosed in U.S. Pat. No. 5,424,078 (issued Jun. 13, 1995); U.S. Pat.
The pharmaceutical compositions of the invention can comprise pharmaceutically acceptable excipients other than emollients, demulcents, and antioxidants such as those listed in REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY 866-885 (Alfonso R. Gennaro ed. 19th ed. 1995; Ghosh, T. K.; et al. TRANSDERMAL AND TOPICAL DRUG DELIVERY SYSTEMS (1997, including, but not limited to, protectives, adsorbents, preservatives, moisturizers, buffering agents, solubilizing agents, skin-penetration agents, and surfactants.

Suitable demulcents include, but are not limited to, benzoin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, and polyvinyl alcohol. Suitable emollients include, but are not limited to, animal and vegetable fats and oils, myristyl alcohol, alum, and aluminum acetate. Suitable preservatives include, but are not limited to, quaternary ammonium compounds, such as benzalkonium chloride, benzethonium chloride, cetrimide, dequalinium chloride, and cetylpyridinium chloride; mercurial agents, such as phenylmercuric nitrate, phenylmercuric acetate, and thimerosal; alcoholic agents, for example, chlorobutanol, phenylethyl alcohol, and benzyl alcohol; antibacterial esters, for example, esters of parahydroxybenzoic acid; and other anti-microbial agents such as chlorhexidine, chlorocresol, benzoic acid and polymyxin.

Suitable antioxidants include, but are not limited to, ascorbic acid and its esters, sodium bisulfite, butylated hydroxytoluene, butylated hydroxyanisole, tocopherols, and chelating agents like EDTA and citric acid. Suitable moisturizers include, but are not limited to, glycerin, sorbitol, polyethylene glycols, urea, and propylene glycol. Suitable buffering agents for use with the invention include, but are not limited to, acetate buffers, citrate buffers, phosphate buffers, lactic acid buffers, and borate buffers.

Suitable solubilizing agents include, but are not limited to, quaternary ammonium chlorides, cyclodextrins, benzyl benzoate, lecithin, and polysorbates. More specifically, camphor is slightly soluble in water, soluble in alcohol, ether, benzene, acetone, oil of turpentine, glacial acetic acid, chloroform, carbon disulphide, solvent naphtha and fixed and volatile oils. It is also soluble in aniline, nitrobenzene, tetralin, decalin, methylhexalin, petroleum ether, higher alcohols, concentrated mineral acids, phenol, liquid ammonia and liquid sulphur dioxide.
Embodiments may include skin-penetration agents such as, but are not limited to, ethyl alcohol, isopropyl alcohol, octylphenylpolyethylene glycol, oleic acid, polyethylene glycol 400, propylene glycol, N-decymethylsulfoxide, fatty esters \( \text{i.e., isopropyl myristate, methyl laurate, glycerol monooleate, and propylene glycol monooleate} \); and N-methyl pyrrolidone.

In treating in the form of a dermal-type patch, the pharmaceutical composition is contained within a laminated structure that serves as a drug delivery device to be affixed to the skin. In such a structure, the pharmaceutical composition is contained in a layer, or "reservoir", underlying an upper backing layer. The laminated structure may contain a single reservoir, or it may contain multiple reservoirs. In one embodiment, the reservoir comprises a polymeric matrix of a pharmaceutically acceptable contact adhesive material that serves to affix the system to the skin during active ingredients delivery. Examples of suitable skin contact adhesive materials include, but are not limited to, polyethylenes, polysiloxanes, polyisobutylanes, polycrylates, polyurethanes, and the like. The particular polymeric adhesive selected will depend on the particular active ingredients, vehicle, etc., \( \text{i.e., the adhesive must be compatible with all components of the active ingredients-containing composition. Alternatively, the active ingredients-containing reservoir and skin contact adhesive are present as separate and distinct layers, with the adhesive underlying the reservoir which, in this case, may be either a polymeric matrix as described above or it may be a liquid or hydrogel reservoir, or may take some other form.} \]

Targeted drug delivery of the pharmaceutical composition, sometimes called smart drug delivery, is a method of delivering medication to a patient in a manner that increases the concentration of the medication in some parts of the body relative to others. The goal of a targeted drug delivery system is to prolong, localize, target and have a protected drug interaction with the diseased tissue, \( \text{i.e., in this case a slow-release transdermal patch} \). The conventional drug delivery system is the absorption of the drug across a biological membrane, whereas the targeted release system releases the drug in a dosage form. The advantages to the targeted release system is the reduction in the frequency of the dosages taken by the patient, having a more uniform effect of the drug, reduction of drug side-effects, and reduced fluctuation in circulating drug levels.

In an embodiment, the active agents are formulated into liposomes for delivery. Liposomes are microscopic spheres made from fatty materials, predominantly phospholipids.
Because of their similarity to phospholipid domains of cell membranes and an ability to carry substances, liposomes can be used to protect active ingredients and to provide time-release properties in medical treatment. Liposomes are made of molecules with hydrophilic and hydrophobic ends that form hollow spheres. They can encapsulate water-soluble ingredients in their inner water space, and oil-soluble ingredients in their phospholipid membranes. Liposomes are made up of one or more concentric lipid bilayers, and range in size from 50 nanometers to several micrometers in diameter. Liposomal formulations have been used for many years to enhance the penetration of topically applied ingredients. Liposomes are made from lecithin, egg or it can be synthesized. These phospholipids can be both hydrogenated and non-hydrogenated. Phosphatidylcholine is extracted from these sources and can be both saturated and unsaturated. Other phospholipids including essential fats like linoleic acid and alpha linolenic acid can be used. Additionally, polyethylene glycol and cholesterol are considered liposomal material because of their lipid structure.

[0114] The active agents can be delivered to cancers such as SCCs in the digestive tract for example, or in another location that is difficult to access, e.g. locally in a slow-release formulation or via an implanted pump. Any formulation that delivers the active agents to the intended site is within the scope of this invention. The literature on making such formulations is well known to those in the art.

E. Dosages and Dosing Frequency

[0115] In the experiments herein, camphor, camphor-oil, and 2-APB were tested. For camphor which can be toxic if ingested at high enough concentrations, the preferred embodiment is topical administration. It has been shown that topical administration of toxic doses of camphor has little effect on elevating systemic amounts. (See D. Martin, et al., J. Clin. Pharmacol, 2004 Oct; 44 (10): 1151-57.) The toxicity of the active agents will have to be determined as a factor affecting their formulation and mode of application. Some agents formulated to have very low toxicity may be able to be administered to cancers inside the body, as opposed to on the skin. Routine experimentation will determine this.

[0116] In 1980, the U.S. Food and Drug Administration set a limit of 11% allowable camphor in consumer products, and totally banned products labeled as camphorated oil, camphor oil, camphor liniment, and camphorated liniment except for "white camphor essential oil" for fear that they would be ingested and become toxic. In the past, when camphor was used medicinally,
the oral doses ranged from 120-300mg (Wade 1977). The parenteral dose range was from 60-200mg (which not recommended any more).

[0117] High doses of camphor (>20%) have been reported to cause skin irritation as well as toxicity when ingested. However, the high doses of camphor described herein for therapeutic use of keratinocyte-derived lesions (going up to 99%) are neither intended for ingestion nor for application on very large areas of the body. Rather, it is intended that camphor for treating cancers or AK is applied locally to affected areas and in doing so it would not cause systemic toxicity even if applied at very high doses. The goal is to treat the patient's cancer, and in doing so doses higher than 11% may be necessary to eliminate or control the cancer or AK. If the cancer is relatively nonaggressive it may respond to a lower dose than would be needed for treating aggressive forms of keratinocyte-derived cancers. In the experiments described herein, therapeutic doses of camphor up to 30% were tested for topical application without systemic toxicity. Ultimately, any side effects at higher doses may be further managed. It is not expected that high dose formulations of camphor or any other active that is potentially toxic if ingested, would be available over the counter. Rather it would be a prescription drug for treating cancer or AK. It may be advisable that very high doses be applied by a physician or under the direction of a physician.

[0118] In Canada, amounts of camphor are approved for over the counter formulations of up to 20% for multiple, daily applications in over the counter preparations. Some high doses of camphor may cause necrosis of normal cells along with killing the cancer; however, there is a cost benefit risk to controlling the cancer even at the expense of killing some normal cells. Moreover the dose can be adjusted once the cancer is under control. Normal cells around the periphery of the lesion that may become necrotic can be removed and healthy skin/cells will grow back.

[0119] It has also been found in the art, that low-dose (i.e., 46.80 mg) dermal application of camphor, results in low plasma concentrations of camphor. Even when four and eight patches were applied for 8 hours, there appeared to be relatively low systemic exposure to the potentially toxic. (See D. Martin, et al., J. Clin. Pharmacol, 2004 Oct; 44 (10): 1151-57.) This supports the position that one trained in dosing and dosing frequency would recognize that higher concentrations of the TRPV3 agonist or derivative thereof may be used, as long any potential side-effects can be separately managed.
[0120] Dosages and dosing frequency will be determined by a trained medical professional depending on the activity of the TRPV3 agonist or derivative thereof, the dose, the particular topical formulation, and the identity and severity of the dermatologic disorder. Camphor applied to the skin of volunteers as a 20% solution in alcohol produced no significant sensation of irritation or pain at normal skin temperatures (Green 1990). As described above, therapeutically effective amounts of camphor range from about 0.0608-99.5%, preferably in the range of 10%-50%, therapeutically effective amounts of camphor-oil range from about 0.0608-99.5%, preferably in the range of 10%-50%, and therapeutically effective amounts of 2-APB 0.00005-5%.

5. Kits

[0121] In other embodiments of the invention, various kits are also provided. Typically, the kits include a pharmaceutical composition as described herein and instructions for the use of the pharmaceutical composition and dosage regime. The kit can comprise the pharmaceutical composition of the invention in a suitable container with labeling and instructions for use. The container can be, but is not limited to, a dropper or tube. The pharmaceutical composition of the invention can be filled and packaged into a plastic squeeze bottle or tube. Suitable container-closure systems for packaging pharmaceutical compositions of the invention are commercially available for example, from Wheaton Plastic Products, 1101 Wheaton Avenue, Millville, N.J. 08332.

[0122] Preferably, instructions are packaged with the formulations of the invention, for example, a pamphlet or package label. The labeling instructions explain how to administer pharmaceutical compositions of the invention, in an amount and for a period of time sufficient to treat or prevent SCC and AK and symptoms associated therewith. Preferably, the label includes the dosage and administration instructions, the topical formulation's composition, the clinical pharmacology, drug resistance, pharmacokinetics, absorption, bioavailability, and contraindications.

6. Methods of Treatment

[0123] Embodiments of the invention provide methods of diagnosing, and treating subjects having keratinocyte-derived lesions, e.g., SCC (including high-risk forms), non-melanoma skin cancers (including high-risk forms), and AK, and subjects at risk of developing non-melanoma skin cancer, SCC, or AK, in a subject.

[0124] Treatment of these conditions preferably involves topical application of
therapeutically effective amounts (amounts that reduce or prevent abnormal keratinocyte proliferation) of one or more active agents. The TRPV3 agonists described herein are collectively referred to as "active agents" The amount of active agent will vary depending on many factors, including the severity of the disease, the size of the lesion, the location of the lesion, the age, sex and immune status of the subject. As described above, in preferred embodiments, therapeutically effective amounts of camphor and camphor oil range from 0.0608-99.5% (wt/vol or vol/vol), preferably in the range of 10-50%, and 2-APB ranges from about 0.00005-0.05%.

[0125] While the amount of the active agent useful in treating an enumerated disease such as SCC, non-melanoma cancers, or AK in vitro is a good starting point for determining an in vivo dose, various factors known to those skilled in the art affect the actual therapeutic amounts used in vivo, especially in humans. In the in vitro experiments described herein, there is no barrier to penetration of the active agent. In vivo mouse studies showed that 10-30% camphor-oil had therapeutic utility in treating SCC. Higher or lower doses may also be effective as is discussed herein in vivo. Penetration of the active agents to the targeted SCC, non-melanoma cancers, or AK cells can be optimized by adjusting the dose, but also by formulating the active agents in ways that enhance uptake, for example by using skin-penetration agents in the formulations. The active agents of the invention should be applied to the lesion in such a way as to treat the margins of the tumor or affected area in addition to the bulk area of the lesion. Some normal cells will thus be contacted with active agent in this process.

[0126] In an embodiment of the invention, the active agents are applied to the affected area having the cancerous lesions for a period of time before the lesion is surgically removed, or for a period of time after is the lesion surgically removed, or more preferably before and after surgery. Application of the active agent before surgery will reduce tumor burden and application after surgery will kill any stray cancer cells left behind and reduce the risk of a recurrence. The present therapies can be used in conjunction with other therapies that are effective in treating an enumerated disease. Preferably, the subject is human.

[0127] High-risk SCCs up until now were determined by the clinician's general impression based on factors described above. SCCs arising in the head and neck area are typically at higher risk for local invasion and metastasis compared to SCCs arising in the trunk and extremities. Locally advanced, aggressive, recurrent or metastatic SCC is much more difficult to treat. SCCs
that arise in the head and neck area, including primary lip, oral cavity, nasal cavity, paranasal sinus, pharyngeal and laryngeal tissues exhibit a local recurrence in 50% of patients and carry an overall survival of only 6-9 months. However, now that it has been discovered that significantly higher or significantly lower expression of one or more of the biomarkers TRPV3, TRPV1, TRPA1, and TRPC1 indicates that the SCC or non-melanoma cancer is a high risk form; methods are provided for making a definitive diagnosis and thereby avoiding over-aggressive treatment of non high risk cancers, and under aggressive treatment of high risk cancers.. It is now possible in view of the new discoveries to diagnosis the high-risk form of SCC or high risk non-melanoma and differentiate it from an ordinary form of SCC or non-melanoma cancer in a patient by determining if a biopsy of the lesion has either significantly higher or significantly lower mRNA encoding one or more of the TRP channels (TRPV3, TRPC1, TRPV1, and TRPA1) compared to the level in a sample of normal tissue. Thus, in some embodiments, methods comprise obtaining a biopsy of the affected area from a patient diagnosed or suspected of having an enumerated disease, and obtaining a control biopsy from either a normal subject or from a matched biopsy of normal tissue from the diagnosed patient In a preferred embodiment, if the level of mRNA encoding one or more of the TRP channels (TRPV3, TRPC1, TRPV1, and TRPA1) in the patient biopsy of the affected area and in the control biopsy are then determined, and if the mRNA level in the patient biopsy of the affected area is significantly higher than the level in the control biopsy, then the diagnosis is made that the patient has a high-risk form of SCC or non-melanoma. Once the diagnosis of high-risk SCC or non-melanoma is confirmed, then it is possible to determine that the patient is in need of more aggressive treatment (e.g., Mohs surgery followed by chemotherapy and radiation).

[0128] Other methods are directed to (i) treating squamous cell carcinoma (SCC) or AK in a patient in need thereof, comprising contacting one or more skin lesions with the pharmaceutical composition; (ii) slowing the progression of benign tumors to cutaneous squamous cell carcinoma (SCC) in a patient in need thereof, comprising contacting one or more skin lesions with the pharmaceutical composition; (iii) promoting regression of pre-malignant skin tumors in a patient in need thereof, comprising contacting one or more skin lesions with the pharmaceutical composition; (iv) and attenuating malignant squamous cell carcinoma (SCC) conversion in a patient in need thereof, comprising contacting one or more skin lesions with the pharmaceutical composition. These methods are useful as an alternative
to surgery for such patients when the lesion is in a sensitive area that is difficult to treat surgically due to the potential for scarring e.g., the face.

[0129] Immunocompromised patients (e.g., organ transplant patients, "OTR") have an increased risk of developing aggressive, high-risk SCC and high-risk non-melanoma cancers, and biopsies from such individuals should routinely be tested to determining if there is a significantly higher or significantly lower level of mRNA encoding one or more of the following biomarkers of high risk SCC/non-melanoma cancer: TRPV3, TRPC1, TRPV1, and TRPA1 to help determine the course of treatment. If an OTR subject does not have abnormal biomarker mRNA levels, the subject may respond to non-aggressive surgical removal of the SCC combined with TRPV3, TRPV1, TRPA1, and TRPC1 agonist therapy, and not require the more aggressive surgery.

PCR

[0130] In certain embodiments, expression of mRNA encoding one or more of the following: TRPV3, TRPC1, TRPV1, and TRPA1 can be determined in a biological sample using known techniques, from which the level of gene expression can be inferred. Levels of mRNA can be quantitatively measured by northern blotting which gives size and sequence information about the mRNA molecules. A sample of RNA is separated on an agarose gel and hybridized to a radioactively labeled probe that is complementary to the target sequence. Or more typically RT-qPCR is used wherein reverse transcription is followed by real-time quantitative PCR (qPCR). Reverse transcription first generates a DNA template from the mRNA; this single-stranded template is called cDNA. The cDNA template is then amplified in the quantitative step, during which the fluorescence emitted by labeled hybridization probes or intercalating dyes changes as the DNA amplification process progresses. With a carefully constructed standard curve, qPCR can produce an absolute measurement of the number of copies of original mRNA, typically in units of copies per nanolitre of homogenized tissue or copies per cell. qPCR is very sensitive. A highly specific RT-qPCR assay for human TRPV3, including well-behaved specific primers, has been developed and is potentially the basis for a diagnostic assay.

[0131] The examples disclose the specific methods used to quantitate TRPV3, TRPV1, TRPA1, and TRPC1 mRNA expression, using the specific primers:

1. TRPV3

   hGAPDH_f: AAG GGC ATC CTG GGC TAC
2. **TRPC1**
   - **hTRPC1**
     - **TRPC1_2_F**
     - CCT TCT GTT AGT GGC TTT TTG C
     (SEQ. ID. NO. 5)

   - **TRPC1_2_R**
     - GCC TAC ATT TGC TGG TCT TCA
     (SEQ. ID. NO. 6)

3. **TRPA1**
   - **hTRPA1**
     - **TRPA1_3_F**
     - GAG AGT CCT TCC TAG AAC CAT ATC TGA
     (SEQ. ID. NO. 7)

   - **TRPA1_3_R**
     - CAT GAG GAC AAT TGG GAC AAA TAT T
     (SEQ. ID. NO. 8)

4. **TRPV1**
   - **hTRPV1**
     - **TRPV1_F**
     - GTT TGG GGG TGT TGG TGT T
     (SEQ. ID. NO. 9)

   - **TRPV1_R**
     - CCT TTG GGA TGT GGT TCT GT
     (SEQ. ID. NO. 10)

**GenBank Accession Numbers:**

1. **TRPV3**
TrpV3 Mouse sequences:
- Gene-TRPV3
- Gene ID: 246788
- NC_000077.6
- TrpV3 mRNA-
- NM_145099.2

TRPV3 human sequences:
- Gene-TRPV3
- Gene ID: 162514
- NG_032144.2
- mRNA-
- NM_001258205.1

2. TRPV1
TrPV1 Mouse sequences:
- Gene-TRPV1
- Gene ID: 193034
- NC_000477
- TrPV1 mRNA-
- NM001001445.1

TRPV1 human sequences:
- Gene-TRPV1
- Gene ID: 7442
- NG_029716
- mRNA-
- NM018727.5

3. TRPA1
TrpA1 Mouse sequences:
- Gene-TRPA1
- Gene ID: 277328
- NC_000067
- TrpA1 mRNA
- NM177781.4

TRPA1 human sequences:
- Gene-TRPA1
- Gene ID: 8989
- NC000008.10
niRNA-
NM007332.2

4. TRPC1
TrpCl Mouse sequences:
Gene-TRPC1
Gene ID: 22063
NC_000075
TrpA1 mRNA
NM01 164312

TRPC1 human sequences;
Gene-TRPC1
Gene ID:7220
NG_030369.1

mRNA-
NM001251845.1

The qPCR cycling conditions are as follows:
48 °C for 30 min
95 °C for 10 min
95 °C 15 sec
60 °C 1 min
Repeat the last two steps 40 times.

[0132] Target nucleic acids are amplified to obtain amplification products. Suitable nucleic acid amplification techniques are well known to a person of ordinary skill in the art, and include polymerase chain reaction (PCR) as for example described in Ausubel et al., Current Protocols in Molecular Biology (John Wiley & Sons, Inc. 1994-1998) (and incorporated herein). The most commonly used nucleic acid amplification technique is the polymerase chain reaction (PCR). PCR is well known in this field and comprehensive description of this type of reaction is provided in E. van Pelt-Verkuil et al., Principles and Technical Aspects of PCR Amplification, Springer, 2008. PCR is a powerful technique that amplifies a target DNA sequence against a background of complex DNA. If RNA is to be amplified (by PCR), it must be first transcribed into cDNA (complementary DNA) using an enzyme called reverse transcriptase. Afterwards, the resulting cDNA is amplified by PCR. PCR is an exponential process that proceeds as long as the conditions for sustaining the reaction are acceptable. The components of the reaction are: (i). pair of primers—short single strands of DNA with around
10-30 nucleotides complementary to the regions flanking the target sequence; (ii). DNA polymerase—a thermostable enzyme that synthesizes DNA; (iii). deoxyribonucleoside triphosphates (dNTPs)—provide the nucleotides that are incorporated into the newly synthesized DNA strand; and (iv). buffer— with MgCl₂ provide the optimal chemical environment for DNA synthesis.

[0133] PCR typically involves placing these reactants in a small tube (~10-50 µl) containing the extracted nucleic acids. The tube is placed in a thermal cycler; an instrument that subjects the reaction to a series of different temperatures for varying amounts of time. The standard protocol for each thermal cycle involves a denaturation phase, an annealing phase, and an extension phase. The extension phase is sometimes referred to as the primer extension phase. In addition to such three-step protocols, two-step thermal protocols can be employed, in which the annealing and extension phases are combined. The denaturation phase typically involves raising the temperature of the reaction to 90-95°C to denature the DNA strands; in the annealing phase, the temperature is lowered to ~50-60°C for the primers to anneal; and then in the extension phase the temperature is raised to the optimal DNA polymerase activity temperature of 60-72°C for primer extension. This process is repeated cyclically around 20-40 times.

[0134] There are a number of variants to the standard PCR protocol such as multiplex PCR, linker-primed PCR, direct PCR, tandem PCR, real-time PCR and reverse-transcriptase PCR, amongst others, which have been developed for molecular diagnostics.

[0135] Multiplex PCR uses multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. By targeting multiple genes at once, additional information may be gained from a single test-run that otherwise would require several experiments. Optimization of multiplex PCR is more difficult though and requires selecting primers with similar annealing temperatures, and amplicons with similar lengths and base composition to ensure the amplification efficiency of each amplicon is equivalent.

[0136] Linker-primed PCR, also known as ligation adaptor PCR, is a method used to enable nucleic acid amplification of essentially all DNA sequences in a complex DNA mixture without the need for target-specific primers. The method firstly involves digesting the target DNA population with a suitable restriction endonuclease (enzyme). Double-stranded
oligonucleotide linkers (also called adaptors) with a suitable overhanging end are then ligated to the ends of target DNA fragments using a ligase enzyme. Nucleic acid amplification is subsequently performed using oligonucleotide primers which are specific for the linker sequences. In this way, all fragments of the DNA source which are flanked by linker oligonucleotides can be amplified.

[0137] Direct PCR describes a system whereby PCR is performed directly on a sample without any, or with minimal, nucleic acid extraction. It has long been accepted that PCR reactions are inhibited by the presence of many components of unpurified biological samples, such as the haem component in blood. Traditionally, PCR has required extensive purification of the target nucleic acid prior to preparation of the reaction mixture. With appropriate changes to the chemistry and sample concentration, however, it is possible to perform PCR with minimal DNA purification, or direct PCR. Adjustments to the PCR chemistry for direct PCR include increased buffer strength, the use of polymerases which have high activity, ability to process, and additives.

[0138] Tandem PCR utilizes two distinct rounds of nucleic acid amplification to increase the probability that the correct amplicon is amplified. One form of tandem PCR is nested PCR in which two pairs of PCR primers are used to amplify a single locus in separate rounds of nucleic acid amplification. The first pair of primers hybridize to the nucleic acid sequence at regions external to the target nucleic acid sequence. The second pair of primers (nested primers) used in the second round of amplification bind within the first PCR product and produce a second PCR product containing the target nucleic acid, that will be shorter than the first one. The logic behind this strategy is that if the wrong locus were amplified by mistake during the first round of nucleic acid amplification, the probability is very low that it would also be amplified a second time by a second pair of primers and thus ensures specificity.

[0139] Real-time PCR, or quantitative PCR, is used to measure the quantity of a PCR product in real time. By using a fluorophore-containing probe or fluorescent dyes along with a set of standards in the reaction, it is possible to quantitate the starting amount of nucleic acid in the sample. This is particularly useful in molecular diagnostics where treatment options may differ depending on the pathogen load in the sample.

RT-PCR

[0140] Typically DNA sequences are amplified, although in some instances RNA sequences can
be amplified or converted into cDNA, such as by using RT PCR. Reverse-transcriptase PCR (RT-PCR) is used to amplify DNA from RNA. Reverse transcriptase is an enzyme that reverse transcribes RNA into complementary DNA (cDNA), which is then amplified by PCR. RT-PCR is widely used in expression profiling, to determine the expression of a gene or to identify the sequence of an RNA transcript, including transcription start and termination sites. It is also used to amplify RNA viruses such as human immunodeficiency virus or hepatitis C virus. "cDNA" or "complementary DNA" is DNA synthesized from a messenger RNA (mRNA) template in a reaction catalyzed by the enzyme reverse transcriptase and the enzyme DNA polymerase. Complementary base sequences are those sequences that are related by the base-pairing rules. In DNA, A pairs with T and C pairs with G. In RNA, U pairs with A and C pairs with G. In this regard, the terms "match" and "mismatch" as used herein refer to the hybridization potential of paired nucleotides in complementary nucleic acid strands. Matched nucleotides hybridize efficiently, such as the classical A-T and G-C base pair mentioned above. Mismatches are other combinations of nucleotides that do not hybridize efficiently.

[0141] A reverse transcriptase PCR™ amplification procedure may be performed when the source of nucleic acid is fractionated or whole cell RNA. Methods of reverse transcribing RNA into cDNA are well known (see Sambrook et al., 1989). Alternative methods for reverse polymerization utilize thermostable DNA polymerases. These methods are described in WO 90/07641. Representative methods of RT-PCR are described in U.S. Pat. No. 5,882,864.

[0142] Other embodiments are set forth in the summary of the invention, or described in the pharmaceutical composition section below.

7. Examples

[0143] The invention is illustrated herein by the experiments described by the following examples, which should not be construed as limiting. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference. Those skilled in the art will understand that this invention may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will fully convey the invention to those skilled in the art. Many modifications and other embodiments of the invention will come to mind in one skilled in the art to which this invention pertains having the benefit of the teachings presented in the foregoing description. Although specific terms are
employed, they are used as in the art unless otherwise indicated.

Example 1: Methods and Materials for *In vitro* Experiments.

[0144] *Cell culture.* Normal human keratinocytes were isolated from human foreskins by undergoing two enzymatic dissociation steps, first in dispase then in trypsin. Cells were cultured in EpiLife supplemented with human keratinocyte growth supplement (HKGS; Invitrogen). For all experiments with undifferentiated cells, keratinocytes were harvested or images at -75% confluency. Differentiation was induced by adding CaCl$_2$ to the EpiLife media to reach a final [Ca$^{2+}$] of 1.2 mM. Cells were typically cultured for 3 days under differentiation conditions before they were assayed. Keratinocytes were cultured for <5 passages. Purified Camphor (CAS #76-22-2), 2-APB (Tocris) or vehicle (1% EtOH)-containing media were used as indicated.

[0145] Organotypic human skin cultures and primary SCC lines were isolated from human SCCs and cultured as previously described (Bachelor *et al.*, 2011). In brief, human fibroblasts were seeded in a collagen matrix on a 70 μm filter insert and incubated at 37°C for 5-7 days. Human keratinocytes or SCC cells were then seeded on the apical surface of the fibroblast-collagen matrix and cultured for 2 days submerged in media to allow monolayer formation. The media was then removed from the apical surface (raising), exposing the keratinocytes or SCC cells to air. The cultures were maintained in this fashion for up to 14 days, and the media to the fibroblasts below was changed every 2-3 days. At the time of raising, 50 μM 2-APB or vehicle (1% EtOH) was added to the media.

[0146] *Histology.* Sections were fixed in 4% paraformaldehyde, paraffin embedded and sectioned at 8 μm. Hematoxylin and Eosin (H&E) stained sections were imaged with a bright field microscope equipped with a 10X (0.3 NA) and 20X (0.4 NA) objectives and an Axiocam color CCD camera (Zeiss AxioObserver.Z1). Epidermal thickness was assessed by measuring the nucleated layers with ImageJ software (NIH ImageJ). Number of invading cells was determined by counting the number of round nuclei below the basement membrane per 10X field (fibroblast nuclei are flatten and were excluded). Four non-serial paraffin sections per experiment were examined. Six random frames per section were quantified.

[0147] *Proliferation Assays.* Cells were treated with 2-APB or camphor for 24 hours at 37°C, exposed to EdU for 45 min, fixed and stained with DAPI for cell counting. EdU Click-It
assays (Invitrogen) were used to assess cell-cycle entry. The cells were then fixed and stained with DAPI. Three fields per well were assayed via a 20X objective and there were 4 wells per treatment in each experiment.

[0148] **Ratiometric Calcium imaging.** Human keratinocytes and SCC cells were washed in Ringer's solution (in mM: NaCl 140, KCl 5, D-glucose 10, HEPES 10, CaCl$_2$ 2, MgCl$_2$ 2, pH 7.2) and loaded with 5 µM fura-2AM and 1 µM pluronic (Invitrogen) for 30 minutes in the dark. During live-cell imaging, saturating concentrations of TRP agonists (10 mM Camphor, 100 µM 2-APB 1 µM Capsaicin, 300 µM Mustard oil or 3 µM 4dPDD) in Ringer's were used. Cells were imaged for 20 s to establish a baseline and then acutely treated with each agonist for 60 seconds. Imaging was performed on an Olympus 1X81 microscope with a 20X (NA 0.17) objective and a Hamamatsu ORCA-R2 camera. 340- and 380-nm excitation and 540-nm emission filters were used to capture fura-2 fluorescence. Data are expressed as a ratio of fluorescent signals (340/380). All data acquisition was performed in MetaFluor. Data analysis was performed with custom algorithms in IgorPro. Cells with baseline levels +2 SD above the mean were excluded from analysis as unhealthy. Responders were designated as cells whose 340/380 ratio was ≥20% above baseline.

[0149] **qPCR.** RNA was isolated using RNeasy Kit (Qiagen) and reversed transcribed. Four technical replicates were run for each primer set and cDNA sample using SYBR Green (Applied Biosystems) for quantitative readout. Complementary DNA was synthesized with oligo-dT primers and Superscript III (Invitrogen). Primers were generated in Primer3. Primer pairs were optimized for qPCR and validated in control specimens. Standardized SYBR green amplification protocols were used on the StepOnePlus ABI machine as suggested by the distributor (Applied Biosystems). Melting curves were generated for all products to confirm a single amplicon for each product. To determine gene expression in each sample, cycle thresholds (CT) of the gene of interest were normalized to the reference gene GAPDH (ACT). Fold change was determined using the AACT method where vehicle or growth conditions were used as the calibrator (AACT=[(CT (target gene) - CT (reference gene)] - [(CT (calibrator) - CT (reference gene))]. All gene amplifications were performed in quadruplicate.
Statistics. Experimental replicates were performed using normal human epidermal keratinocytes independently isolated from different human neonatal foreskin specimens (n=14 specimens). For each independent experiment, 3-8 technical replicates were performed. Twelve individual SCC and two normal skin biopsises were obtained. Four technical replicates were performed on each biopsy. Organotypic cultures were carried out in triplicate. Data are expressed as means +SEMs unless noted. Statistical significance was assessed with Chi-square analysis, unpaired two-tailed Student's t tests or two-way ANOVA with Bonferroni post hoc analysis (GraphPad Prism).

Example 2: Responses to TRP-channel agonists are potentiated in differentiating human keratinocytes in vitro.

TRP channels are selectively activated by endogenous and exogenous ligands at micromolar to millimolar concentrations. Since TRP channel activity increases over this range to allow graded Ca$^{2+}$ influx, receptor activity levels can be monitored by live-cell Ca$^{2+}$ imaging. A ratiometric calcium indicator (Fura-2) was employed to determine whether a panel of TRP-channel agonists elicited cytoplasmic calcium increases in human epidermal keratinocytes. (FIG. 1, FIG. 2, and Table 1). Normal human keratinocytes were cultured in low-calcium media to promote proliferation or in 1.2-mM calcium media to induce differentiation. For live-cell imaging, keratinocytes were then washed and bathed in Ringer's solution containing 2mM calcium so that calcium signals could be directly compared between cell populations.

Consistent with previous reports, it was determined that addition of a selective TRPV4 agonist, 4a-phorbol-12,13 didecanoate (4dPDD), elicited robust increases in intracellular Ca$^{2+}$ in >70% of keratinocytes cultured in growth or differentiation conditions (Kida et al., 2012). By contrast, the proportion of cells showing Ca$^{2+}$ increases in response to camphor, which activates TRPV3 (Moqrich et al., 2005; Vanden abele et al., 2003) and TRPV1 (Xu et al., 2005) and inhibits TRPA1 (Sawada et al., 2007; Vanden Abeele et al., 2003) was 5.7-fold greater in differentiated keratinocytes than in proliferating keratinocytes (FIG. 1(A)-(C) and Table 2, $\chi^2<0.0001$). The magnitude of camphor-evoked responses was also significantly larger in differentiated keratinocytes (differentiated=78+1% increase over baseline, growth=55+1% increase, P<0.001, Student's t test).

To narrow camphor's molecular target, additional agonists of TRPV1, TRPA1, and TRPV3 were tested to determine whether these channels were functional in normal human
epidermal keratinocytes. Keratinocytes displayed little or no change in cytoplasmic calcium in response to saturating concentrations of TRPV1 or TRPA1 agonists (TRPV1, 1 μM capsaicin=2.3+/−2.6% of cells responding; TRPA1, 300 μM mustard oil=0.6+/−1.2% of cells responding; FIG. 2(A) and Table 2). Capsaicin and mustard oil are high efficacy agonists that robustly activate their respective TRP-channel targets (Table 1). Therefore, proliferating human keratinocytes have only low levels of functional TRPV1 or TRPA1 in vitro.

These results were confirmed using a higher affinity TRPV3 agonist that does not target TRPV1 or TRPA1, 2-APB, which is structurally distinct from camphor and activates TRPV3 at a different binding site (FIG. 1(D); (Chung et al., 2004a; Hu et al., 2009). Like camphor, 2-APB elicited cytoplasmic calcium increases in keratinocytes and these 2-APB-evoked responses were upregulated in differentiated keratinocytes. The proportion of cells responding to either TRPV3 agonist was significantly increased in differentiated keratinocytes (FIG. 1(D), cell type P<0.05, two-way ANOVA). Although 2-APB has been reported to block TRPC1 channels (Chung et al., 2004a), TRPC1 inhibition cannot account for these results because channel block will cause cytoplasmic calcium decreases rather than the calcium increases observed. The 2-APB-evoked Ca^{2+} increases observed were consistent with Ca^{2+}-channel activation rather than inhibition. It was determined that calcium signals elicited by camphor and 2-APB were increased in human keratinocytes upon differentiation.

It was then determined whether these increased 2-APB or camphor-evoked activity reflect enhanced TRP-channel gene expression. Quantitative PCR (qPCR) demonstrated that TRPV3 transcripts were enriched 66-fold in differentiated keratinocytes compared with those cultured in growth conditions (FIG. 1(E), FIG. 2(B); P=0.04). TRPV1 and TRPC1, which were expressed at lower levels than TRPV3, were also upregulated in differentiated keratinocytes (FIG. 2.2(B)). By contrast, TRPA1 was only amplified at detectable levels in one out of three biological replicates. TRP channel upregulation was accompanied by increased expression of well-established early differentiation markers including keratin-1 (KRT1), loricin (LOR), filaggrin (FLG; FIG. 1(E); (Li et al., 1995). Therefore, differentiation stimulates TRP-channel gene expression in normal keratinocytes, which results in an increase in both the proportion of keratinocytes responding to 2-APB and camphor and the magnitude of these cytoplasmic calcium signals.

Example 3: Camphor and 2-APB exert, pleotropic effects on human keratinocyte behavior in vitro.
As Ca\textsuperscript{2+} triggers the commitment switch from proliferation to differentiation in keratinocytes, it was reasoned that increasing intracellular calcium by incubating keratinocytes in TRP agonists might be sufficient to induce this cell-fate switch in low-calcium growth media. To test this hypothesis, normal human keratinocytes were cultured for 24 hours under low-Ca\textsuperscript{2+} conditions in the presence of low (12.5 µM), half-maximal (50 µM) and saturating (100 µM) concentrations of 2-APB (FIG. 3(A)) or camphor (0-8 mM) (FIG. 3(E)). These TRP agonists were chosen because they reliably elicited intracellular calcium signaling in normal human keratinocytes (FIG. 1).

First, cellular morphology, cell counts and cell cycle entry were assessed as evidenced by EdU incorporation (FIG. 3(A)-(C)). At 12.5 or 50 µM 2-APB, keratinocyte morphology was indistinguishable from vehicle-treated cells. At 12.5 µM 2-APB, a population increase was noted in one set of primary keratinocytes (325+/−5% compared with vehicle-treated controls, n=4 replicates, P<0.0001; Student's t test) and a significant increased in EdU incorporation was also noted across all experiments (FIG. 3(B); P<0.05, Student's t test). Low concentrations of 2-APB promote keratinocytes proliferation. At 50 µM, 2-APB caused a slight reduction in cell number and completely inhibited EdU incorporation, indicating cell-cycle arrest (FIG. 3(A)-(C)). Similar effects were observed with 4-8 mM camphor (FIG. 3(C)). As camphor can activate both TRPV3 and TRPV1 channels, these experiments were repeated in the presence of a specific TRPV1 antagonist, AMG-9810 (Gavva et al., 2005). TRPV1 inhibition did not alter camphor's effects on keratinocyte proliferation (FIG. 4). Therefore, camphor-evoked cell-cycle arrest is unlikely to be mediated through TRPV1-dependent mechanisms. At 100 µM, 2-APB induced necrotic morphological changes and a loss of cell numbers, consistent with a previous report that saturating 2-APB concentrations induce keratinocyte cytotoxicity (FIG. 3(A)-(B) and Table 1; Borboiro et al., 2011).

2-APB exerts activity-dependent, pleotropic effects on keratinocyte behavior. Moreover, they identify 2-APB and camphor concentrations (50 µM and 4-8 mM, respectively) that arrest proliferation without inducing necrotic changes in normal human keratinocytes. As proliferation arrest is a hallmark of cell-fate commitment, this observation is consistent with the hypothesis that TRP agonists can induce the fate switch from proliferation to differentiation in human keratinocytes.

It was then determined whether moderate TRPV3 activation, specifically,
incubation with 50 µM 2-APB, was sufficient to promote expression of keratinocyte differentiation genes. In low-calcium growth media, LOR and FLG transcript levels were induced more than 15-fold in keratinocytes treated for 24 hours with 50 µM 2-APB compared with vehicle controls (FIG. 3(D)). This observation is consistent with reduced LOR protein observed in TRPV3 knock-out mice. By comparison, it was observed that little change in expression of the late-stage differentiation genes involucrin (IVL) and transglutaminase 3 (TGM3; FIG. 3(D)). This might be due to the brief 2-APB incubation period, as late differentiation markers are typically not expressed in culture until 48 hours after the induction of keratinocyte differentiation (Hennings et al., 1980). Alternatively, 2-APB might preferentially regulate early differentiation genes. Collectively, it was determined that constitutive treatment with 50 µM 2-APB in low-calcium media was sufficient to commit human keratinocytes to a differentiated state.

Since 50 µM 2-APB induced proliferation arrest and keratinocyte differentiation in vitro, it was predicted that 2-APB might alter epidermal stratification. This prediction was tested with human organotypic 3D skin equivalent models (Commandeur et al., 2009; Obrigkeit et al., 2009). Human keratinocytes were seeded on dermal matrices, allowed to form a monolayer, raised to an air-liquid interface to induce stratification and then treated for 7 days with either vehicle or 50 µM 2-APB. 2-APB-treated skin equivalents displayed a one-third reduction in nucleated epidermal layers (FIG. 3(E)-(F)). These data extended observations in two-dimensional keratinocyte cultures by demonstrating that constitutive exposure to 2-APB altered human keratinocyte behavior in a stratifying epidermis.

Example 4: TRP-channel expression is dysregulated in high-risk human cutaneous SCCs in vitro.

SCC is a pathological condition marked by perturbed keratinocyte differentiation and alterations in EGFR signaling. As TRPV3 potently activates EGFR signaling in mouse keratinocytes (Cheng et al., 2010; Pan et al., 2011; Tajeddine & Gailly, 2012), it was reasoned that TRP channel expression might be dysregulated in SCC. To test this hypothesis, expression levels of TRP-channel genes were quantified in high-risk human SCC specimens (FIG. 5(A) and FIG. 6). It was determined that TRPV3, TRPV1, TRPA1, and TRPC1 gene expression levels were significantly dysregulated in >60% of SCC of SCC biopsies compared with normal human skin (FIG. 5(A) and FIG. 6). By comparison, expression of signature SCC biomarkers Cyclin D1 (CCND1) and EDFR (Hardisson, 2003)
was altered in only 17% and 41% of patient specimens, respectively (FIG. 6). Therefore, TRP channel expression is dysregulated in a majority of human SCCs examined.

[0161] To ask whether TRP channels are functional in human SCC keratinocytes, two cell lines derived from human SCC tumors (SCC-13 and SCC-39; (Bachelor et al., 2011); FIG. 5(B) were assayed with live-cell calcium imaging. Camphor-evoked cytoplasmic calcium signals were increased in SCC-13 cells (FIG. 5(B) compared with proliferating keratinocytes (FIG. 1(C)). Consistent with enhanced camphor-evoked responses, expression levels of TRPV3, LOR, and FLG were augmented in SCC-13 cells (FIG. 5(C). Expression levels of TRPV1, TRPC1, and TRPA1 were also increased (FIG. 7). By contrast, SCC-39 cells showed significantly fewer camphor-evoked calcium responses compared with SCC-13 cells (FIG. 5(B)). Similarly, SCC-39 cells showed reduced expression levels of TRPV3, TRPV1, TRPC1, TRPA1, LOR and FLG compared with normal keratinocytes and SCC-13 cells (FIG. 5(B and FIG. 7). Together, these results illustrated that human SCCs preserve the coordinated pattern of expression between TRP channels and differentiation genes that we observe in normal human keratinocytes (FIG. 1(E)). Moreover, their responses to camphor mirror their respective levels of TRP channel expression.

[0162] 2-APB reduces SCC tumor growth and invasion in human preclinical models. As TRP-channels are expressed in human SCC keratinocytes and TRP agonists arrest keratinocyte proliferation, it was reasoned that TRP agonists might be candidates for SCC-targeted therapy. To test this notion, organotypic human skin cultures were seeded with SCC cells. SCC-39 showed significantly more cells invading into the dermis (FIG. 5(D)-(E) and FIG. 8B) than SCC-13 (FIG. 4; P=0.003, Student's t test). SCC-39's enhanced invasiveness was consistent with its less differentiated molecular signature (FIG. 3(C); (Ratushny et al., 2012). Conversely, SCC tumor formation apical to the dermis was significantly larger in SCC-13 compared with SCC-39 (FIG. 5(E), FIG. 8C, and FIG. 9). A third cell line, SCC-73, displayed a much more dysplastic nature with no obvious basement membrane formation (FIG. 8A). Thus, in 3D organotypic cultures, these SCC cell lines recapitulate a range of tumor behaviors observed in vivo.

[0163] 2-APB treatment dramatically reduced tumorigenesis in organotypic cultures seeded with each of these three SCC cell lines. In SCC-39 organotypic cultures, 2-APB treatment inhibited cell invasion by >93% compared with vehicle-treated cultures (P<0.0001, Bonferroni
post hoc, FIG. 5(E)). Tumor formation above the dermis in SCC-13 and SCC-39 was reduced by $>37\%$ (P<0.01, Bonferroni post hoc, FIG. 5(E)). 2-APB also dramatically reduced the invasive nature and growth of SCC-73 tumor cells in human 3D cultures (FIG. 8A, N=3 culture pers treatment). Together, these human preclinical models support the conclusion that 2-APB reduces SCC tumor size and dermal invasion in vitro.

Example 5: Methods and Materials for In vivo Experiments.

[0164] **Animals.** Animal use was conducted according to guidelines from the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and the Institutional Animal Care and Use Committee of Columbia University Medical Center.

[0165] **Experimental design.** The overall experimental design is summarized in FIG. 10.

[0166] **Chemical carcinogenesis model.** Genetically identical, age-matched, female FVB mice (N=24; Jackson Laboratories) were used because they are susceptible to skin carcinogenesis with this model. All topical agents were applied to shaved dorsal skin. To ensure that topical agents permeated the skin barrier, all topical agents were dissolved in acetone. Mice (6-7 weeks of age) were shaved once on the dorsal surface with electric clippers. After two days, animals were checked to ensure that they did not show signs of hair regrowth, confirming that they were in the telogen (resting) stage of the hair cycle. Each animal received a single topical application of 400 nmol DMBA in 200 $\mu$ acetone to initiate tumorigenesis. One week later, mice received twice weekly applications of 10 nmol TPA in 200 $\mu$ acetone for a period of 15 weeks. All mice developed multiple skin tumors at 15 weeks with this protocol (median: 14 tumors per mouse, range: 3-30 per mouse).

[0167] **Tumor quantification.** Tumor number and location were documented weekly. Tumor diameters were estimated using digital calipers. A lesion was classified as a papilloma based on its appearance as a non-ulcerated, fleshy pedunculated or sessile wart-like mass with a diameter in any dimension $\geq$2 mm that persisted for at least one week. In this chemical carcinogenesis model, a subset of high-risk papillomas will convert to malignant SCCs. Lesions were classified as malignant SCCs based on the following criteria: 1) conversion from a fleshy lesion to a flattened circular growth with a depressed center, 2) spontaneous ulceration (Allen et al, 2003; PMID: 12566297). Mice were monitored daily and euthanized when they reached one of the following IACUC-approved endpoints: 1) a tumor $>20$ mm in diameter in any dimension, 2)
tumor ulceration that penetrates below the dermis, leading to loss of skin barrier, 3) signs of anemia for ≥24 h, 4) tumor burden that interferes with eating or drinking (e.g., on the mouth), or 5) gross appearance indicating distress (hunched posture, lethargy, persistent recumbence). Tissue from euthanized animals was harvested for histology and molecular analysis. Lungs and lymph nodes were examined for SCC-derived metastases.

[0168] Camphor oil or vehicle (acetone) treatment. Three days after the last TPA treatment, mice were randomly assigned to Camphor oil (synthetic camphor white oil CAS# 8008-51-3 Sigma Aldrich) and control treatment groups, which were matched for total papilloma burden (N=162 lesions in 12 mice per group). Mice assigned to the Camphor oil group were treated daily with topical 20% camphor white oil in acetone (w/w; 400 µl applied drop wise to papilloma and SCC lesions; CAS 8008-51-3; Sigma catalog # W223115; lot MKBG8153V). Mice assigned to the control (acetone vehicle) group were treated daily with topical acetone (400 µl applied drop wise to lesions).

[0169] Histopathological Analysis. Skin tumors were surgically excised from euthanized mice, fixed and embedded in paraffin wax blocks. Histological tumor sections were generated from paraffin blocks and stained with hematoxylin and eosin for microscopic examination. Criteria used for verifying skin lesions as papillomas or SCCs were as described (Bogovski, P. Tumours of the skin. In: V. Turusov and U. Mohr (eds.). Pathology of Tumours in Laboratory Animals).

[0170] Data analysis and statistics. Four measures of tumor burden and survival were assessed: 1) mean number of benign tumors (papillomas) per mouse, 2) total number of malignant SCCs per group, 3) tumor incidence, expressed as the percentage of mice with pre-malignant or malignant lesions, and 4) time to experimental endpoint for each mouse. Data are expressed as mean ± SEM unless noted. Statistical significance was assessed with two-way ANOVA followed by Bonferroni post hoc analysis when appropriate (GraphPad Prism version 5). Tumor burden at different time points within a group were compared with Student's t test (two-tailed). Kaplan-Meier survival analysis was performed to determine if endpoint curves differed. Statistical significance of endpoint curves was assessed with Mantel-Cox and Gehan-Bresholw-Wilcoxon Tests (GraphPad Prism version 5).

[0171] Western blot analysis of keratinocyte differentiation markers. To determine whether topical camphor treatment promotes keratinocyte differentiation in vivo, a separate cohort of
adult female FVB mice were treated twice daily for a period of 5 days with increasing concentrations of topical camphor (0, 15%, 20% or 30% camphor in acetone; N=3-4 mice per group). One day after the final topical dose, mice were sacrificed and epidermal lysates were prepared for immunoblot analysis according to standard protocols (Owens et al., J. Invest. Dermatol. 1996). Epidermal lysates were subjected to western blot analysis to measure levels of keratin 10 (K10, a keratinocyte terminal differentiation marker) and beta-tubulin (a house keeping gene used to normalize samples for protein loading). Protein levels were detected by peroxidase activated luminol exposure to X-ray film and estimated by densitometry using NIH-Image J software. Levels of K10 were normalized to levels of beta-tubulin for each sample; therefore, data are expressed as the ratio of K10 to beta-tubulin (K10/beta-tubulin). Mean protein levels between experimental groups were compared with Student's t tests (one-way, unpaired).

Example 6: Experiments in vivo with a mouse model of SCC

[0172] In order to test camphor oil's effect on SCC tumor burden, a two-stage chemical-induced carcinogeneis mouse model (DMBA-TPA) (FIG. 10) was used to induce benign papillomas and SCCS in adult female mice.

[0173] Daily topical treatment with 20% camphor oil in acetone (w/w) was well tolerated by mice, although hyperkeratosis and slowed hair growth were observed in this group compared with vehicle-treated control mice. The effects on camphor oil on hair growth and hyperkeratosis are consistent with TRPV3 activation, as mutations that cause constitutive TRPV3 activation lead to hyperkeratotic lesions in humans (Olmsted syndrome; Danso-Abeam et al, 2013, PMID: 23692804) and hairlessness in mice (Xaio et al, 2008 PMID: 17706768). Camphor oil treatment caused a dramatic reduction in tumor burden in mice compared with vehicle-treated control mice (N=12 mice per group). This reduction was observed in all three measures of tumor burden that were assessed (FIGs. 11-16).

[0174] Mean number of pre-malignant tumors (FIGs 11-12). Both treatment groups were matched for the mean tumor burden prior to treatment (FIG. 11). Five days before treatment, mice assigned to the camphor-oil group displayed 13.5+2.0 tumors per mouse (mean+SEM; N=12) and those assigned to the control group had 13.5+2.2 tumors per mouse (N=12). The mean number of papillomas per mouse did not differ significantly over time in the control group (week 13: 8.3+3.3 tumors per mouse, P=0.29; Student's t test, two-tailed). By contrast, the
mean number of papillomas per mouse decreased six-fold in the camphor-oil group (week 13: 2.2+1.1 tumors per mouse, P<0.001; Student's t test, two-tailed). A two-factor ANOVA showed a highly significant effect of treatment group on tumor persistence, with camphor-oil treated mice displaying fewer papillomas [F(15,265)=101.40, P<0.0001]. The effect of treatment week was also highly significant [F(15,265)=5.16, P<0.0001]. Finally, there was a significant interaction effect between treatment group and treatment duration [F(15,265)=2.30, P=0.004]. Strikingly, post hoc analysis demonstrated that the tumor burden was significantly reduced in camphor-oil treated mice compared with control mice after only three weeks of once-daily treatment (FIG. 11). Reduction in tumor burden is illustrated in a photomontage of a representative mouse from each treatment group over seven weeks of treatment (FIG. 12). Thus, it was demonstrated that topical camphor oil treatment promotes regression of pre-malignant skin tumors.

Number of malignant SCCs (FIGs 13-15). Mice treated with camphor oil for 13 weeks developed 2.5-fold fewer SCCs than vehicle-treated control mice (camphor-oil group: 9 SCCs; control group: 23 SCCs; FIG. 13). Note that one mouse randomly assigned to the camphor-oil group developed a malignant SCC before camphor oil-treatment began. A two-factor ANOVA indicated a highly significant effect of treatment group on the number of malignant SCCs [F(1,15)=10.20, P=0.006]. The effect of treatment time was also significant [F(15,15)=2.74, P=0.030]. Thus, this analysis indicates that camphor oil slows the progression of benign tumors to SCC.

Along with developing fewer SCCs, mice treated with camphor oil displayed apparent regressions of a subset of early-stage SCCs (FIG. 14). Such regressions were never observed in vehicle-treated control mice. When advanced SCCs developed, they progressed to experimental endpoints in both treatment groups. Histopathological examination of regressed SCCs revealed microscopic areas of residual tumor that resembled SCC in situ. Residual lesions in camphor oil-treated mice displayed intact fascia and muscle layers (FIG. 15, right panel). By contrast, advanced SCCs invaded the fascia and muscle layers (FIG. 15 left and middle panels). These results indicate that daily camphor-oil treatment can suppress SCC proliferation and invasion although it may not completely eradicate SCC lesions. These data indicate that camphor-oil treatment dramatically attenuates malignant SCC conversion in this carcinogenesis model.

Tumor incidence (FIG. 16). At the end of the 15-week TPA treatment period, 100% of
mice in both groups had tumors. Tumor incidence remained at 100% in control mice but decreased to as low as 50% in camphor oil-treated mice (FIG. 16). A two-factor ANOVA showed a highly significant effect of treatment group on tumor incidence [F(1,15)=14.40, P=0.0018]. The effect of treatment day was not significant [F(15,15)=1.0, NS]. Therefore, camphor-oil treatment is sufficient to completely clear tumors in a subset of animals in this carcinogenesis model.

[0178] Endpoint curves (FIG. 17). After 13 weeks of treatment, 75% of control mice and 50% of camphor oil-treated mice had reached an experimental endpoint; however, Kaplan-Meier survival analysis indicated that endpoint curves did not differ significantly between treatment groups (P>0.53, Mantel-Cox and Gehan-Breslow-Wilcoxon tests; FIG. 17). Specific allowances for a survival endpoint were not incorporated into initial experimental design. The effects of camphor oil were tested on the progression of pre-established skin tumors. Relatively high doses of DMBA and TPA were used to increase overall skin tumor burden and the rate of malignant conversion (SCC formation). This approach was utilized in order to generate a large number of pre-malignant tumor targets for camphor treatment. This increased the statistical power of any anti-tumor effects observed following camphor treatment. The utility of this approach is appreciated in FIGs 11-16. Animal welfare guidelines require that animals be euthanized based on the endpoints of any single tumor and do not discriminate between animals with multiple malignant SCCs. Although control mice were routinely euthanized with multiple SCC lesions, camphor mice exhibited a marked reduction in SCC formation (FIG. 13) yet were required to be euthanized along a similar time frame due to the endpoints of a single SCC as opposed to multiple SCCs observed in control mice. Camphor oil treatment would dramatically increase survival under an experimental design where the overall tumor burden was lower (e.g., when control mice sustain a maximum of 1-2 SCCs). Overall, camphor oil treatment is highly effective at reducing the rate of malignant conversion of benign epidermal lesions to SCCs in vivo. Strikingly, camphor oil treatment appears to lead to dramatic regression of pre-established neoplastic skin lesions and these changes in tumor regression could be observed on a daily basis (FIGs 12 & 14). These findings are consistent with in vitro results described herein, which show that camphor blocks proliferation and that 2-APB induces the expression of markers of terminal differentiation in cultured human keratinocytes. Therefore, the anti-tumor effects of camphor may be due to its ability to block neoplastic proliferation in vivo and shift transformed cells to
commit to terminal differentiation.

Example 7: Topical Camphor Treatment Promotes Keratinocyte Differentiation *In vivo*

[0179] *Western blot analysis (FIG. 18).* To determine whether topical camphor treatment promotes keratinocyte differentiation *in vivo*, adult female FVB mice (n=3-4 per group) were treated twice daily for a period of 5 days with increasing concentrations of topical camphor (15%, 20% or 30%) or vehicle only. Whole cell epidermal lysates were generated from each group and subjected to western blot analysis to detect the levels of the terminal differentiation marker cytokeratin, Keratin 10 (K10). Expression of K10, a keratinocyte terminal differentiation marker, increased with camphor treatment in a dose-dependent manner (FIG. 18). Mice treated with 30% camphor displayed significantly higher K10 protein levels compared with mice treated with lower camphor concentrations or vehicle alone (P=0.04; Student's *t* test). It was determined that topical camphor treatment upregulates levels of a keratinocyte terminal differentiation marker *in vivo*. These data confirm and extend *in vitro* studies of human keratinocytes as described herein.

[0180] In the present specification, the invention has been described with reference to specific embodiments thereof. It will, however, be evident that various modifications and changes may be made thereto without departing from the broader spirit and scope of the invention. Accordingly, all such variations and modifications are within the scope of the appended claims.

[0160] The specification and drawings are, accordingly, to be regarded in an illustrative rather than a restrictive sense. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference as if set forth herein in their entirety, except where terminology is not consistent with the definitions herein. Although specific terms are employed, they are used as in the art unless otherwise indicated.
REFERENCES

All citations (e.g., scientific journal publications, patents, and other reference material) mentioned herein are hereby incorporated herein by reference to the same extent as if each individual citation was specifically and individually indicated to be incorporated by reference.


Ophthalmol Vis Sci 52: 485-493. 3053292 3053292


CLAIMS

1. A method comprising:
   (i) identifying a subject having a keratinocyte-derived lesion, comprising squamous cell carcinoma, high-risk squamous cell carcinoma, non-melanoma skin cancer, high-risk non-melanoma skin cancer, and actinic keratosis,
   (ii) administering to the subject a therapeutically effective amount of a TRPV3 agonist or derivative thereof, or a combination of one or more TRPV3 agonists or derivatives thereof, thereby treating or preventing the keratinocyte-derived lesion.

2. The method of claim 1, wherein the TRPV3 agonist is 2-APB or a derivative thereof, or a monoterpene selected from the group consisting of: camphor, camphor-oil, (+)-Borneol, (-)-Isopinocamphone, (-)-Fenchone, (-)-Trans-pinocarveol, Isoborneol, (+)-Camphorquinone, (-)-alpha-Thujone, alpha-pinene oxide, 1,8-Cineole, (-)-alpha-Pinene, Isobornyl acetate, 6-tert-butyl-m-cresol, Carvacrol, Thymol, p-xylenol, Kreosol, Propofol, p-cymene, carvacrol methyl ether, dihydrocarveol, (-)-Carveol, (-)-Isopulegol, (-)-Menthol, (-)-Carvone, (+)-Dihydrocarvone, (-)-Menthone, (+)-Limonene, Terpineol, (+)-Linalool, Geraniol, l-Isopropyl-4-methyl-bicyclo[3.1.0]hexan-4-ol, (-)-alpha-Bisabolol and mugetanol or a derivative thereof.

3. The method of claim 2, wherein the camphor derivative thereof is selected from the group consisting of: 4-methyl-benzylidene camphor [3-(4'-methyl)benzylidene-bornan-2-one], 3-benzylidene camphor[3-benzylidene-bornan-2-one], polyacrylamidomethylbenzylidene camphor [N-[2(4)-2-oxiborn-3-ylidene-methyl]benzyl]acrylamide polymer}, trimonium-benzylidene camphor sulfate[3-(4'-trimethylammonium)-benzylidene-bornan-2-one methyl sulfate], terephthalydene dicamphorsulfonic acid [3,3'(1,4-phenylenedimethine)-bis(7,7-dimethyl-2-oxo-bicyclo[2.2.1]hepta- ne-l-methanesulfonic acid} or salts thereof, and benzylidene camphorsulfonic acid [3-(4'-sulfo)benzylidenebornan-2-one] or salts thereof and norcamphor.

4. The method of claim 2, wherein the amount of camphor ranges from 4-8 mM.

5. The method of claim 2, wherein the amount of camphor ranges from 0.0608%-99.5%.
6. The method of claim 2, wherein the amount of camphor ranges from 10%-50%.

7. The method of claim 2, wherein the amount of camphor oil ranges from 0.0608%-99.5%, preferably 10%-50% (wt/wt).

8. The method of claim 2, wherein the amount of 2-APB ranges from 25 μM to 50 μM.

9. The method of claim 2, wherein the amount of 2-APB ranges from 0.00005-5% (wt/wt or wt/vol).

10. The method of claim 1, wherein the TRPV3 agonist is applied directly to the keratinocyte-derived lesion before it is surgically removed.

11. The method of claim 1, wherein the agonist is applied after surgery directly to the affected area from which the keratinocyte-derived lesion was surgically removed.

12. The method of claim 1, wherein the subject is human.

13. A method comprising:

   (i) obtaining a biopsy of squamous cell carcinoma or non-melanoma cancer from a patient;

   (ii) obtaining a control biopsy either from a normal subject not afflicted with squamous cell carcinoma or non-melanoma cancer and not having an endogenous TRP mutation, or from a matched-sample taken from a non-affected area from the patient;

   (iii) determining an expression level of mRNA encoding one or more TRP ion channels selected from the group consisting of: TRPV3, TRPV1, TRPC1, and TRPA1, in the patient biopsy and in the control biopsy; and

   (iv) diagnosing the squamous cell carcinoma as a high-risk form of squamous cell carcinoma or the non-melanoma cancer as a high risk form of non-melanoma cancer if the level of mRNA encoding the one or more of the ion channels in the patient biopsy is either significantly higher or significantly lower than the corresponding mRNA level in the control biopsy.
14. The method of claim 13, wherein if a diagnosis of a high-risk form of squamous cell carcinoma or high-risk form of non-melanoma cancer is made, then determining that the patient is in need of treatment appropriate for the high-risk form of squamous cell carcinoma or the high-risk non-melanoma cancer.

15. The method of claim 14, wherein the treatment is surgery to remove the high-risk squamous cell carcinoma or high-risk non-melanoma cancer in combination with administering to the patient therapeutically effective amounts of one or more TRPV3 agonists or derivatives thereof to the SCC before and after it is removed.

16. The method of claim 14, wherein one or more TRPV3 agonists or derivatives thereof are applied directly to the squamous cell carcinoma or high-risk non-melanoma cancer.

17. The method of claim 13, wherein the patient is an immunocompromised patient.

18. The method of claim 17, wherein the immunocompromised patient is an organ transplant patient.

19. A pharmaceutical composition comprising therapeutically effective amounts of one or more TRPV3 agonists selected from the group consisting of 2-APB or a derivative thereof, and a monoterpene selected from the group consisting of camphor, camphor-oil, (+)-Borneol, (-)-Isopinocamphene, (-)-Fenchone, (-)-Trans-pinocarveol, Isoborneol, (+)-Camphorquinone, (-)-alpha-Thujone, alpha-pinene oxide, 1,8-Cineole, (-)-alpha-Pinene, Isobornyl acetate, 6-tert-butyl-m-cresol, Carvacrol, Thymol, p-xylene, Kreosol, Propofol, p-cymene, carvacol methylether, dihydrocarveol, (-)-Carveol, (-)-Isopulegol, (-)-Menthol, (-)-Carvone, (+)-Dihydrocarvone, (-)-Menthone, (+)-Limonene, Terpineol, (+)-Linalool, Geraniol, 1-Isopropyl-4-methyl-bicyclo[3.1.0]hexan-4-ol, (-)-alpha-Bisabolol and mugetanol or a derivative thereof.

20. The pharmaceutical composition of claim 19 wherein camphor is dissolved in ethanol.

21. The pharmaceutical composition of claim 19 wherein camphor oil is dissolved in
acetone.

22. The pharmaceutical composition of claim 19 formulated into liposomes.

23. The pharmaceutical composition of claim 19 formulated for topical application.

24. A kit containing the pharmaceutical composition of claim 19.

25. A sunscreen comprising one or more TRPV3 agonists selected from the group consisting of 2-APB or a derivative thereof, and a monoterpenic selected from the group consisting of camphor, camphor-oil, (+)-Borneol, (-)-Isopinocampeol, (-)-Fenchone, (-)-Trans-pinocarveol, Isoborneol, (+)-Camphorquinone, (-)-alpha-Thujone, alpha-pinene oxide, 1,8-Cineole, (-)-alpha-Pinene, Isobornyl acetate, 6-tert-butyl-m-cresol, Carvacrol, Thymol, p-xylenol, Kreosol, Propofol, p-cymene, carvacrol methylether, dihydrocarveol, (-)-Carveol, (-)-Isopulegol, (-)-Menthol, (-)-Carvone, (+)-Dihydrocarvone, (-)-Menthone, (+)-Limonene, Terpineol, (+)-Linalool, Geraniol, l-Isopropyl-4-methyl-bicyclo[3.1.0]hexan-4-ol, (-)-alpha-Bisabolol and mugetanol or a derivative thereof.

26. A method of treating squamous cell carcinoma or actinic keratinosis in a patient in need thereof, comprising contacting one or more keratinocyte-derived skin lesions with the pharmaceutical composition of claim 19.

27. A method of slowing the progression of benign tumors to squamous cell carcinoma or actinic keratinosis in a patient in need thereof, comprising contacting one or more skin lesions with the pharmaceutical composition of claim 19.

28. A method of promoting regression of pre-malignant skin tumors in a patient in need thereof, comprising contacting one or more skin lesions with the pharmaceutical composition of claim 19.

29. A method of attenuating malignant squamous cell carcinoma or actinic keratinosis,
camphor conversion in a patient in need thereof, comprising contacting one or more skin lesions with the pharmaceutical composition of claim 19.
FIG. 7
FIG. 11
FIG. 14
FIG. 15

ACETONE ADVANCED cSCC

CAMPHOR OIL ADVANCED cSCC

CAMPHOR OIL REGRESSED cSCC

200 μm
FIG. 17
<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>TARGETED TRP CHANNELS</th>
<th>ACTION</th>
<th>EFFECTIVE CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-APB</td>
<td>TRPV3</td>
<td>ACTIVATION</td>
<td>EC50 = 28 μm, RANGE = 3.2 - 320 μm</td>
</tr>
<tr>
<td></td>
<td>TRPC1</td>
<td>INHIBITION</td>
<td>IC50 = 68 μM, RANGE = 1 - 10 μM</td>
</tr>
<tr>
<td></td>
<td>ORAI1</td>
<td>INHIBITION</td>
<td>IC50 = 24.5 ± 15.7 nM, RANGE = 7 - 100 μM</td>
</tr>
<tr>
<td></td>
<td>TRPV3</td>
<td>ACTIVATION</td>
<td>EC50 = 119 ± 1 μM, RANGE = 10 nM - 1 μM</td>
</tr>
<tr>
<td></td>
<td>TRPV1</td>
<td>ACTIVATION</td>
<td>EC50 = 211 ± 9 nM, RANGE = 50 nM - 1 μM</td>
</tr>
<tr>
<td></td>
<td>TRPA1</td>
<td>INHIBITION</td>
<td>IC50 = 10 nM - 1 μM, RANGE = 90 nM - 1 μM</td>
</tr>
<tr>
<td></td>
<td>TRPV4</td>
<td>ACTIVATION</td>
<td>IC50 = 24.5 ± 15.7 nM, RANGE = 7 - 100 μM</td>
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<tr>
<td></td>
<td>AMG-9810</td>
<td>ACTIVATION</td>
<td>IC50 = 24.5 ± 15.7 nM, RANGE = 7 - 100 μM</td>
</tr>
<tr>
<td></td>
<td>MUSTARD OIL</td>
<td>ACTIVATION</td>
<td>IC50 = 24.5 ± 15.7 nM, RANGE = 7 - 100 μM</td>
</tr>
<tr>
<td></td>
<td>4μPDD</td>
<td>ACTIVATION</td>
<td>IC50 = 24.5 ± 15.7 nM, RANGE = 7 - 100 μM</td>
</tr>
<tr>
<td>AGONIST</td>
<td>CONCENTRATION</td>
<td>% RESPONDERS (UNDIFFERENTIATED) MEAN±SEM (?) OR SD (?)</td>
<td>N =</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------</td>
<td>--------------------------------------------------</td>
<td>-----</td>
</tr>
<tr>
<td>4αPDD</td>
<td>3 µm</td>
<td>84.8 ± 1.6%[?][7]</td>
<td>3 EXP</td>
</tr>
<tr>
<td>CAMPHOR</td>
<td>10 mM</td>
<td>6.5 ± 2.8%[?][7]</td>
<td>3 EXP</td>
</tr>
<tr>
<td>2-APB</td>
<td>100 µm</td>
<td>2.5 ± 0.9%[?][7]</td>
<td>3 EXP</td>
</tr>
<tr>
<td>CAPSAICIN</td>
<td>1 µm</td>
<td>2.3 ± 2.6%[8]</td>
<td>13 WELLS</td>
</tr>
<tr>
<td>MUSTARD OIL</td>
<td>300 µm</td>
<td>0.6 ± 1.2%[8]</td>
<td>17 WELLS</td>
</tr>
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</table>

**TABLE 2**
<table>
<thead>
<tr>
<th>Box No.</th>
<th>Nucleotide and/or amino acid sequence(s) (Continuation of item I.c of the first sheet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:</td>
</tr>
<tr>
<td>a.</td>
<td>(means)</td>
</tr>
<tr>
<td>□</td>
<td>on paper</td>
</tr>
<tr>
<td>☑</td>
<td>in electronic form</td>
</tr>
<tr>
<td>b.</td>
<td>(time)</td>
</tr>
<tr>
<td>☑</td>
<td>in the international application as filed</td>
</tr>
<tr>
<td>□</td>
<td>together with the international application in electronic form</td>
</tr>
<tr>
<td>□</td>
<td>subsequently to this Authority for the purposes of search</td>
</tr>
<tr>
<td>2.</td>
<td>In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.</td>
</tr>
<tr>
<td>3.</td>
<td>Additional comments:</td>
</tr>
</tbody>
</table>
### INTERNATIONAL SEARCH REPORT

#### Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. **Claims Nos.:**
   - because they relate to subject matter not required to be searched by this Authority, namely:

2. **Claims Nos.:**
   - because they relate to parts of the international application that do not comply with the prescribed requirements so that no meaningful international search can be carried out, specifically:

3. **Claims Nos.:**
   - because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

#### Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

**Group 1:** Claims 1-12 and 19-29 drawn to a method of identifying a subject and treating the subject with a TRPV agonist, and pharmaceutical compositions comprising TRPV agonists.

**Group 2:** Claims 13-18, drawn to a method of diagnosing a squamous cell carcinoma or non-melanoma cancer.

—please see continuation on extra sheet—

1. **[x]** As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. **[ ]** As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. **[ ]** As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. **[x]** No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos. 1-12 and 19-29

#### Remark on Protest

- **[ ]** The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- **[ ]** The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- **[ ]** No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)
INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/70833

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 31/25, A61K 31/69 (2013.01)
USPC - 514/692, 514/64

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/692, 514/64; 424/739, 435/6.14 (keyword limited - see terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Scholar, Search Terms: TRPV3 or OLMS or VRL3; agonist or activator; keratinocyte; lesion or cancer or carcinoma; squamous cell carcinoma

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>US 2010/0136533 A1 (HWANG et al.) 03 June 2010 (03.06.2010) entire document, especially paras [0007]; [0010]; [0017]; [0031]; [0045]; [0048]; [0050]; [0059]</td>
<td>1-9, 19-20, 24, 26</td>
</tr>
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<td></td>
<td>10-12, 21-23, 25, 27-29</td>
</tr>
<tr>
<td>Y</td>
<td>US 201 1/0196043 A1 (MUHAMMAD et al.) 11 August 2011 (11.08.2011) entire document, especially paras [0008]; [0031]; [0211]; [0244]</td>
<td>10-12, 27</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation of other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"A" document member of the same patent family

Date of the actual completion of the international search
03 April 2014 (03.04.2014)

Date of mailing of the international search report
23 APR 2014

Name and mailing address of the ISA/US
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P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer:
Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)
Continuation of Box No. III Observations where unity of invention is lacking

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I requires a TRPV3 agonist and methods of treating a subject with a TRPV3 agonist, not required by Group II.

Group II requires method steps of diagnosing a squamous cell carcinoma or non-melanoma cancer, not required by Group I.

Common Technical Features

The feature shared by Groups I and II is the association of TRPV3 with squamous cell carcinoma or non-melanoma cancer. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by US 2010/0136533 A1 to Hwang et al. (hereafter ‘Hwang’). Hwang discloses that an agonist of TRPV3 (para [0030], FPP is an agonist of TRPV3), may be used to treat non-melanoma cancer (para [0048]. "The present invention also provides a method for treating skin disease containing the step of administering a pharmaceutically effective dose of FPP to a subject", para [0050] - "The skin disease herein is resulted from wound healing and over-proliferation of cells, which is selected from the group consisting of ... basal cell carcinoma"). As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I and II therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.