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(54) Title: COMPOSITIONS AND METHODS COMPRISING SIRTUINS

(57) Abstract: The present technology relates to cosmetic and pharmaceutical compositions useful for controlling the rate of cell destruction and minimizing the appearance of aging. In particular, the present technology relates to compositions and methods related to a combination of a sirtuin activator with a sirtuin-offsetting agent.

TITLE

Compositions and Methods Comprising Sirtuins

BACKGROUND

[0001] The present technology relates to cosmetic and pharmaceutical compositions useful for controlling the rate of cell destruction and minimizing the appearance of aging. In particular, the present technology relates to compositions and methods comprising a combination of a sirtuin activator with a sirtuin-offsetting agent.

[0002] Sirtuins are a class of protein deacetylase enzymes that require nicotaminde adenine dinucleotide (NAD) as a cofactor in deacetylating lysine residues in target proteins. Acetylation and deacetylation of certain amino acids is a post-translational modification that controls the activities of some target proteins. The targets that were initially discovered were the histone proteins that package DNA in cell nuclei; thus, these enzymes are commonly referred to as histone deaceteylases (HDACs), despite the fact that many non-histone target proteins have been discovered subsequent to the initial discoveries.

[0003] Sirtuins have been found to influence various biological phenomena, including cellular stress responses such as DNA repair, replicative senescence and apoptosis (suicide or "programmed cell death" response that cells typically undergo following serious or irreparable damage). Specifically, sirtuins have been found to delay apoptosis of damaged cells, thereby slowing down or eliminating their destruction.

[0004] There is concern that the mechanism through which sirtuins may slow down aging may possess an inherently dangerous side effect – specifically, the apoptic response protects organisms by eliminating damaged cells that could otherwise become genetically unstable and lose normal growth controls and proper differentiated function. By having the effect of delaying apoptosis of damaged cells, sirtuins might allow them to escape normal

checks, resulting in overgrowth of tissues with abnormal progeny cells, which would compromise proper tissue function and possibly even imperil organism survival.

[0005] Thus, a need exists for "safe" sirtuin compounds and methods – specifically, compositions that harness the anti-aging benefits of sirtuins without the undesirable side effects; as well as methods of optimizing such compositions and of using them in anti-aging applications for patients.

SUMMARY OF THE DISCLOSED TECHNOLOGY

[0006] In certain embodiments, the present technology is directed to compositions comprising a sirtuin activator (also referred to herein as a sirtuin stimulator) as well as a sirtuin-offsetting agent.

[0007] In certain embodiments, the present technology is directed to methods of formulating a composition, or of optimizing the efficacy of a composition for a patient, or of optimizing cell maintenance in a patient, the methods comprising selecting a sirtuin stimulator having a known quantitative prolonging effect on a cell, and selecting a sirtuin-offsetting agent having a known quantitative opposite effect on the cell, and optimizing the balance between the two effects based on a known desired ultimate effect on the cell.

[0008] In certain embodiments, the present technology is directed to a method of prolonging the life of a cell and simultaneously avoiding proliferation of cell damage, the method comprising the steps of: stimulating Sirt1 activity in the cell; and inhibiting a non-sirtuin HDAC in the cell.

[0009] In certain embodiments, the present technology is directed to methods of treating a patient, or methods of reducing the appearance of aging in a patient, or methods of optimizing cell maintenance in a patient, comprising applying a composition including sirtuin stimulator and a sirtuin-offsetting agent to the body of a patient.

BRIEF DESCRIPTION OF THE DRAWING

[0010] FIG. 1 shows the experimental results of testing regarding the ability of prototype formulation to enhance the apoptotic response of human keratinocytes that had been exposed to DNA-damaging UV radiation.

DETAILED DESCRIPTION

[0011] Sirtuin function appears to be affected by metabolic state. More broadly, sirtuins appear to help individual cells (and therefore organisms overall) to survive stress, likely by effecting a delay in the apoptotic response, so as to allow cells the time and opportunity to repair whatever stress-related damage they may suffer. It is presumed that any lengthening of lifetime of a cell that results from enhanced sirtuin activity is a consequence of delayed apoptosis of cells under stressful conditions. The cells of greatest interest in this regard would be stem cells. It is believed that the anti-aging effect of sirtuins is at least partly dependent on maintaining the regenerative capacity of such cells and of the tissues they support.

[0012] However, as mentioned above, there is concern that the anti-aging mechanism(s) of sirtuins may present an inherent danger. For example, they may impair the normal apoptic response that would otherwise eliminate damaged cells, and that by doing so, could encourage overgrowth of abnormal cells and tissues. Further, molecular consequences of sirtuin stimulation, such as down regulation of the tumor suppressor gene p53, may also be a cause for concern about enhancing sirtuin function.

[0013] In order to offset the negative effects of sirtuins, "sirtuin-offsetting" agents have been proposed. As used herein, a "sirtuin-offsetting" agent (or "sirtuin-offsetter") is one that at least partly counteracts the effects of sirtuin as it relates to prolonging cell life. Examples of "sirtuin-offsetting" agents will be discussed in greater detail herein. For example, it has been discovered herein that the decreased activity of certain non-sirtuin

HDACs may provide a safeguard. In particular, in certain embodiments of the technology described herein, it has been shown that Class 1, 2 and 4 HDACs have a fundamentally different molecular mechanism and different overall biological effects from the sirtuins, which are Class 3 HDACs. These "non-sirtuin" HDACs do not use NAD as a co-substrate. They also differ from the sirtuins in other ways, *e.g.*, in terms of their target proteins and regulation. Anti-aging or life extension effects have not been demonstrated as consequences of stimulation of non-sirtuin HDACs.

[0014] It has been found herein that these HDACs can be targeted for anti-inflammatory benefits and to prevent proliferation of genetically damaged or unstable cells. Non-sirtuin HDAC inhibitors (HDACi's) have been found to be effective at inhibiting proliferation and promoting differentiation or apoptosis and may be useful for cancer treatment, because, among other reasons, in cancer cells many important genes are abnormally repressed by extreme levels of histone deacetylation; thus, the genes that would otherwise be controlling proliferation and initiating differentiation or apoptosis may be inactivated by the non-sirtuin HDACs.

[0015] Therefore, in certain embodiments, the present technology is directed to compositions that both stimulate Sirt1 activity (the human sirtuin that is the homolog of yeast Sir2) in order to obtain anti-aging benefits, and also simultaneously inhibit sirtuin-offsetting agents such as, *e.g.*, the non-sirtuin HDACs to the extent sufficient to avoid poorly controlled growth of damaged cells that could eventually compromise tissue function.

[0016] In certain embodiments, the compositions herein provide the dual effect of sirtuin stimulation and inhibition of HDAC (protein (histone) deacetylases).

[0017] Thus, in certain embodiments, the relative amounts of sirtuin (or sirtuin stimulator) and sirtuin-offsetting agent in a composition according to the present embodiments is balanced in order to optimize the cell maintenance of the patient. As used

herein, "cell maintenance" means the balance of prolonging the life of cells without over-prolonging that can lead to proliferation of damaged tissue and harm to the patient.

[0018] In certain embodiments, the present technology provides methods for optimizing the preservation of body cells by balancing the Sirt1 activity of a sirtuin composition with the inhibitory activity of an HDACi.

[0019] Data have been developed herein that demonstrate, in *in vitro* studies, the ability to inhibit preferentially the growth of cells that are genetically damaged by exposure to UV light using a formulation that includes activity for inhibiting non-sirtuin HDACs. Cells were subjected to a sub-lethal dose of UV, but by applying a composition in accordance with certain embodiments herein, inhibition of further growth was shown. The effect was to provide time for the cells to repair, and if not, hold them in a quasi-senescent state until they expired.

[0020] In certain embodiments, the compositions herein may comprise either a sirtuin itself, or a sirtuin stimulator, for example, resveratrol. In certain embodiments, the compositions are particularly useful for applying to the skin of a patient.

[0021] The compositions discussed herein may be in any form that can be applied to the body of a patient; for example, to the skin. In certain embodiments, they may be cosmetically or pharmaceutically acceptable forms that can be incorporated into lotions, creams, sprays, gels, serums, liquids, suspensions or the like. Encapsulation technologies such as liposomes, micellar constructs and the like are also contemplated.

CLAIMS

What is claimed:

1. A composition comprising:

   (a) a sirtuin stimulator; and

   (b) a sirtuin-offsetting agent.

2. The composition of claim 1, wherein the amounts of (a) and (b) are selected to optimize the cell maintenance in a patient.

3. The composition of claim 1, wherein (b) comprises a non-sirtuin histone deacetylase inhibitor (HDACi).

4. A cosmetic composition comprising:

   (c) a first composition that comprises a sirtuin stimulator; and

   (d) a second composition that comprises a sirtuin-offsetting agent.

5. The composition of claim 4 in the form of a gel, cream, spray, suspension, liquid, paste or lotion.

6. A method of formulating a composition, the method comprising:

   (a) selecting a sirtuin or sirtuin stimulator having a known quantitative prolonging effect on a cell's life; and

   (b) selecting a sirtuin-offsetting agent having a known quantitative opposite effect on the cell's life; and

   (c) optimizing the balance between the two effects based on a known desired

ultimate effect on the cell.

7. A method of prolonging the life of a cell and simultaneously avoiding proliferation of cell damage, the method comprising the steps of:

(a) stimulating Sirt1 activity in the cell; and

(b) inhibiting a non-sirtuin HDAC in the cell.

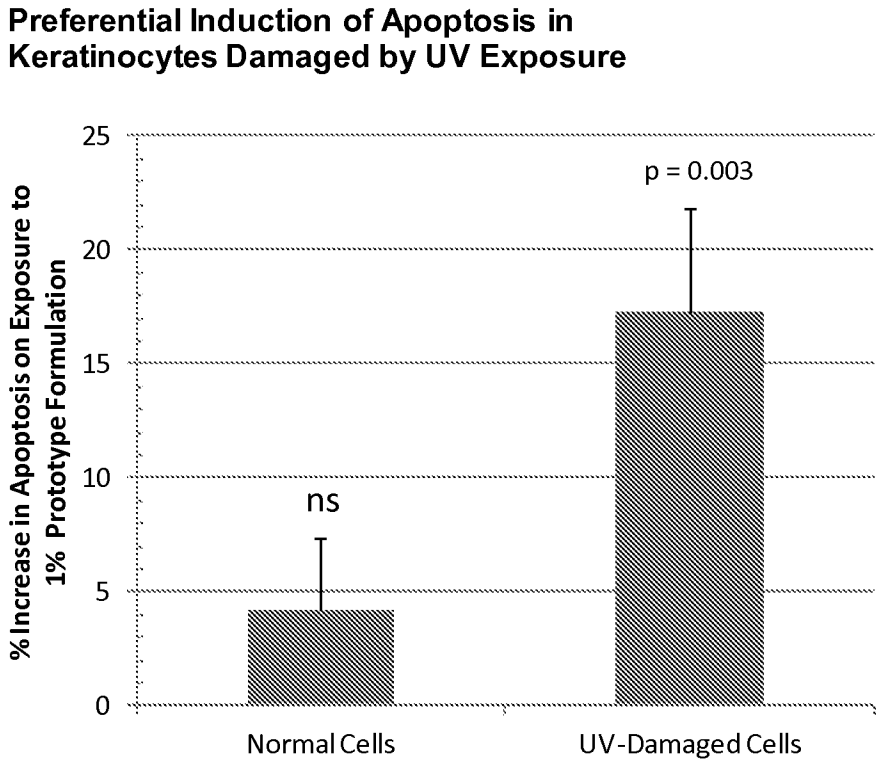
Preferential Induction of Apoptosis in Keratinocytes Damaged by UV Exposure
% Increase in Apoptosis on Exposure to 1% Prototype Formulation
25
20
15
10
5
0
ns
p = 0.003
Normal Cells
UV-Damaged Cells

FIGURE 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/19971

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61Q 19/08; A61K 8/64; A61K 8/66 (2015.01)
CPC - A61Q 19/08; A61K 8/64; A61K 8/66; A61K 31/465
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)- A61Q 19/08; A61K 8/64; A61K 8/66 (2015.01)
CPC- A61Q 19/08; A61K 8/64; A61K 8/66; A61K 31/465

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/401; 514/844; 424/94.1; 514/734 Patents and NPL (classification, keyword; search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Databases: Google Scholar, Google Patent, PatBase
Search terms used: cosmetic, skin care, anti-aging, sirtuin, sirtuin-stimulator, resveratrol, sirtuin-offsetting, HDAC inhibitor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/0017341 A1 (Gourlaouen) 16 January 2014 (16.01.2014) Abstract; para [0009]-[0010], [0057]-[0058], [0148]-[0149]; Example 1-4.	1-2 and 4-6
Y		3 and 7
Y	US 2013/0102009 A1 (Dai et al.) 25 April 2013 (25.04.2013) para [0234].	3 and 7
A	US 2005/0096256 A1 (Sinclair) 05 May 2005 (05.05.2005) Example 1-4.	1-7
A	US 2010/0144885 A1 (Pandey) 10 June 2010 (10.06.2010) para [0020]-[0029].	1-7

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"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 or 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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 April 2015 (28.04.2015)	08 JUN 2015

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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