1. (WO2012106692) COMPOUND AND METHOD FOR INCREASING TELOMERE LENGTH

COMPOUND AND METHOD FOR INCREASING TELOMERE LENGTH

[0001] CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of United States Provisional Patent Application 61/439409, filed February 4, 2011.

[0003] FIELD OF THE INVENTION

[0004] The present invention relates to methods and compositions for increasing telomere length by increasing telomerase activity in cells.

[0005] BACKGROUND OF THE INVENTION

[0006] Telomerase is a ribonucleoprotein that catalyzes the addition of telomeric repeats to the ends of telomeres. Telomeres are long stretches of repeated sequences that cap the ends of chromosomes and are believed to stabilize the chromosome. In humans, telomeres are typically 7-10 kb in length and comprise multiple repeats of the sequence -TTAGGG-.

Telomerase is not expressed in most adult cells, and telomere length decreases with successive rounds of replication. After a certain number of rounds of replication, the progressive shortening of the telomeres results in the cells entering a telomeric crisis stage, which in turn leads to cellular senescence. Certain diseases are associated with rapid telomeric loss, resulting in premature cell senescence. Expression of the gene encoding the human telomerase protein in human cells has been shown to confer an immortal phenotype, presumably through bypassing the cells' natural senescence pathway. In addition, expression of the telomerase gene in aging cells with short telomeres has been shown to produce an increase in telomere length and restore a phenotype typically associated with younger cells.

[0007] Somatic cells, in contrast to tumor cells and certain stem cells, have little or no telomerase activity and stop dividing when the telomeric ends of at least some

chromosomes have been shortened to a critical length, leading to programmed cellular senescence (cell death). Since the loss of telomeric repeats in somatic cells, leading to senescence, is augmented by low telomerase activity, induction of telomerase activity, which has the effect of adding arrays of telomeric repeats to telomeres, thereby imparts to mortal somatic cells increased replicative capacity, and imparts to senescent cells the ability to proliferate and appropriately exit the cell cycle upon repair of damaged tissue.

[0008] Methods of increasing telomerase activity therapeutically have been investigated by, for example, Bodnar Science 279(5349):349-52 (Jan. 16 1998)); White, PCT Int. Appl. Pubn. No. WO 2000/08135 (Feb. 2000)); Hannon et al. PCT Int. Appl. Pubn. WO 99/35243 (July 1999) and PCT Int. Appl. Pubn. No. WO 2000/031238 (June 2000)); Franzese et al. Lifescience 69(13) 1509-20 (2001), and Yudoh et al. J. Bone and Mineral Res. 16(8): 1453-1464 (2001). In these reports, telomerase activity is generally increased by overexpression of hTERT, the gene encoding the protein component of human telomerase, or by expression of proteins which mediate assembly of telomerase, e.g. heat shock proteins (White, PCT No. WO2000/08135). Franzese et al. reported that Saquinavir, a protease inhibitor prescribed for treatment of HIV infection, increased telomerase activity in peripheral blood mononuclear cells; Vasa et al. Circ Res. 87(7) 540-2 (2000) described activation of telomerase, and a resulting delay in endothelial senescence, by administration of a nitric oxide (NO) precursor.

[0009] Various saponins of the astragalo side family have been reported as having various biological effects including increasing telomerase activity, Harley et al. PCT Int Appl. Pubn. No. WO2005/000245. Increase in telomerase activity in cells has been shown to result from administration of compounds isolated from astragalus membranaceus root in US Published Application 2010/0292197 and in US Patent 7,846,904, both licensed to Geron Corporation. It would be beneficial to develop a compound which was an effective telomerase activator.

[0010] SUMMARY OF THE INVENTION

[0011] The invention described herein is generally related to compounds and methods for increasing telomerase activity in cells and compositions for use in such methods. Such methods and compositions may be used on cells in cell culture, i.e. in vitro or ex vivo, or in vivo, such as cells growing in tissues of a subject, including human subjects and non-human mammals.

[0012] Silymarin, also known as milk thistle extract, is a complex of flavonolignans known to inhibit hepatitis C virus infection and also displays antioxidant, anti-inflammatory and immunomodulatory actions, Polyak, et al, PNAS, March 30, 2010, Vol. 107 No. 13, 5995-99. Based on these characteristics, Silymarin was explored as a telomerase activator. The seven known flavonolignans, silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, and silydianin, and the flavonoid taxifolin are also investigated.

[0013] Additional experimentation showed potential telomerase activation by horny goat weed (Epimedium sagittatum), Grape Seed (Vitis vinifera), Turmeric (Curcuma longa), Bacopa (Bacopa monnieri), Pomegranate (Punica granatum), DL-alpha lipoic acid, Asian ginseng, (Panax ginseng), Green Tea, White Tea, Black Tea (Camellia sinensis), Acacia (Acacia nilotica), Plantain (Plantago major), L-glutathione, Velvet Bean (Mucuna pruriens), Hawthorn (root) (Crataegus pinnatifida), Quercetin, Boswellia, (Boswellia serrata), Maca

(Lepidium meyenii), Hawthorn (fruit) (Crataegus pinnatifida), Resveratrol, Harada (Terminalia chebula), Shilajit, Chia (Salvia hispanica), N-Curcusorb (trade name for version of Turmeric), Polygonum Cuspidatum (trans resveratrol), pterostibene, (a

synthetic form of resveratrol developed by ChronaDex Company), Tumipure (trade name for Turmeric ingredient by Naturex Company).

[0014] Based on in vitro experimental studies, a formulation of a compound for activating telomerase and increasing measured telomere length in humans was developed.

[0015] EXPERIMENTAL RESULTS FOR TELOMERASE INDUCTION

[0016] An outside laboratory, Sierra Sciences of Reno, Nevada, was engaged to screen samples to determine induction of hTERT mRNA in normal human BJ fibroblast cells.

Composition of the samples was not disclosed to the laboratory, and test results were recorded by sample number.

[0017] Each sample was diluted in DMSO to create a 50.00 mg/mL stock solution. After DMSO addition, each stock solution was sonicated for 2 minutes and centrifuged at 291 x g for 1 minute. An aliquot of the supernatant from each 50.00 mg/mL stock solution was taken and diluted in an 8-point dilution series (n=8, d=3, CH=50.00 mg/mL, CL=0.02 mg/mL). 1.00 of each dilution point was used to treat 10,000 (or 2,000 for CV90) BJ cells in triplicate with 150 of cell media in each well.

[0018] Final treatment concentrations were: 333.33 µg/mL; 111.11 µg/mL; 37.04 µg/mL; 12.35 µg/mL; 4.12 µg/mL; 1.37 µg/mL; 0.46 µg/mL; and 0.15 µg/mL. A 96-well format was implemented using 10,000 cells per well for hTERT RT-PCR and CV24 (24 hour treatment). 2,000 cells per well were used for CV90 (90 hour treatment).

[0019] When RT-PCR produced two or more replicates at a particular concentration wherein the number of induced hTERT transcripts was greater than zero, the sample was considered an hTERT Positive Sample (Hit). An initial run of 30 samples

yielded two Hits, and a second run of 15 samples yielded four Hits. Results of positive samples is as follows:

[0020] JWA 009230011 (C0316639) [Silymarin at 80% concentration] Three positive replicates at 111.11 µg/mL, with an average hTN of 47.34 (4-fold less than C0057684).

[0021] JWA 92510024 (C0316656) [Silymarin at 80% concentrate using varied extraction method] Two positive replicates at 111.11 µg/mL, with an average hTN of 5.56 (16-fold less than C0057684).

[0022] JWAl 00510-32 (C0316679) [Silymarin extract at 80%] Two positive replicates at 111.11 µg/mL, with an average hTN of 12.50 (13-fold less than C0057684) and a standard deviation of 15.84.

[0023] JWAl 00510-37 (C0316684) [Resveratrol purified extract] Two positive replicates at 37.04 µg/mL, with an average hTN of 7.05 (24-fold less than C0057684) and a standard deviation of 6.86; two positive replicates at 111.11 µg/mL, with an average hTN of 42.43 (4-fold less than C0057684) and a standard deviation of 48.09.

[0024] JWAl 00510-39 (C0316686) [Silymarin extract 78% concentration spry dried] Three positive replicates at 111.11 µg/mL, with an average hTN of 66.85 (2.5-fold less than

C0057684) and a standard deviation of 13.10.

[0025] JWA100510-41 (C0316688) [Silymarin at 75% from crude milk thistle] Two positive replicates at 111.11 µg/mL, with an average hTN of 64.03 (2.5-fold less than C0057684) and a standard deviation of 64.45.

[0026] Follow-on testing of additional samples was undertaken using the same methodology. Hits were obtained on samples containing by horny goat weed (Epimedium sagittatum), Grape Seed (Vitis vinifera), Turmeric (Curcuma longa), Bacopa (Bacopa monnieri), Pomegranate (Punica granatum), DL-alpha lipoic acid, Asian ginseng, (Panax ginseng), Green Tea, White Tea, Black Tea (Camellia sinensis), Acacia (Acacia nilotica), Plantain (Plantago major), L-glutathione, Velvet Bean (Mucuna pruriens), Hawthorn (root) (Crataegus pinnatifida), Quercetin, Boswellia, (Boswellia serrata), Maca (Lepidium meyenii), Hawthorn (fruit) (Crataegus pinnatifida), Resveratrol, Harada (Terminalia chebula), Shilajit, Chia (Salvia hispanica), N-Curcusorb (trade name for version of Turmeric), Polygonum Cuspidatum (trans resveratrol), pterostibene, (a synthetic form of resveratrol developed by ChronaDex Company), Tumipure (trade name for Turmeric ingredient by Naturex Company).

[0027] A recent report by researchers examining the effect of silymarin in protecting endothelial progenitor cells (EPCs) from rapamycin-induced senescence, however, has produced encouraging indication that in that environment silymarin increased telomerase activity in the cells. Parzonko and Naruszewicz, Silymarin Inhibits Endothelial Progenitor Cells ' Senescence and Protects Against the Antiproliferative Activity of Rapamycin:

Preliminary Study J Cardiovasc Pharmacol, Volume 56, No. 6, December 2010. There, incubation of EPCs with silymarin increased telomerase, and coincubation of EPCs with silymarin and rapamycin resulted in counteraction of the diminishing effect known to be caused by rapamycin.

[0028] Based on the test results, silymarin is identified as a significant potential telomerase inducer. Regular ingestion by an individual of effective amounts of silymarin extracted from milk thistle is expected to reverse telomere shortening in the individual and produce longer telomeres.

[0029] COMPOSITION FOR ENHANCING TELOMERE LENGTH

[0030] Based on the experimental evidence of potential induction of telomerase, and expectation of consequent regeneration of telomeres thereby, a combination of ingredients was developed for ingestion to enhance telomere length.

[0031] Milk thistle (Silybum marianum) seed extract, lOmg to 5 grams twice daily. Expected effects of silymarin are set forth in the discussion above.

[0032] Horny goat weed (Epimedium sagittatum) extract, 1 mg to 1 gram twice daily. This is a Hit ingredient.

[0033] Grape seed (Vitis viniferd) extract, 1 mg to 500 mg twice daily. This is a Hit ingredient.

[0034] Turmeric {Curcuma longa) root extract, 1 mg to 1000 mg twice daily. A Hit ingredient.

[0035] Ashwagahnda {Withania somnifera) root extract, 1 mg to 2 grams twice daily. Ashwagandha is an adaptogen, a metabolic regulator which increases the ability of an organism to adapt to environmental factors, and to avoid damage from such factors. Assists in environmental exposure, physiological (external) such as injury or aging, or psychological (internal) such as anxiety. An adaptogen has a normalizing effect, i.e. counteracting or preventing disturbances to homeostasis brought about by stressors.

[0036] Bacoba {Bacoba monnieri) leaf extract, 1 mg to 500 mg twice daily. A Hit ingredient.

[0037] N-acetyl-L-cysteine, 1 mg to 500 mg twice daily. N-acetyl Cysteine extends the replicative lifespan of human cells grown in culture by being a substrate for glutathione synthesis and the increase levels of glutathione reduced free radicals and reduced the rate of accelerated telomere shortening. Acetylcysteine is the N-acetyl derivative of the amino acid L-cysteine, and is a precursor in the formation of the antioxidant glutathione in the body. The thiol (sulfhydryl) group confers antioxidant effects and is able to reduce free radicals.

[0038] Pomegranate {Punica granatum) fruit extract, 2 mg to 25 grams twice daily. A Hit ingredient.

[0039] DL-alpha lipoic acid, 2 mg to 500 mg twice daily. Included as an antioxidant to reduce damage to telomeres; exhibits known characteristics to prevent organ dysfunction, reduce endothelial dysfunction and improve albuminuria, support the cardiovascular system, reduce iron overload, treat metabolic syndrome, improve or prevent age-related cognitive dysfunction, prevent or slow progression of AD, prevent erectile dysfunction and migraine, and reduce oxidative stress and inflammation.

[0040] Asian ginseng {Panax ginseng) root extract, 1 mg to 1 gram twice daily. Hit ingredient.

[0041] Berberine (Coptis chinensis) rhizome extract, 1 mg to 500 mg twice daily. Hit ingredient.

[0042] Bilberry (Vaccinium myrtillus) fruit extract, 1 mg to 1 gram twice daily. Included as an antioxidant.

[0043] Blueberry {Vaccinium angustifolium) fruit extract, 1 mg to 1 gram twice daily. Included as an antioxidant.

[0044] Red raspberry (Rubus idaeus) fruit extract, 1 mg to 1 gram twice daily. Included as an antioxidant.

[0045] Green tea (Camellia sinensis) leaf extract, 1 mg to 1500 mg twice daily. Hit ingredient.

[0046] White tea (C sinensis) leaf extract, 1 mg to 1500 mg twice daily. Hit ingredient.

[0047] Black tea (C sinensis) leaf extract, 1 mg to 1500 mg twice daily, Hit ingredient.

[0048] Acacia (Acacia nilitica) bark extract, 1 mg to 1000 mg twice daily. Hit ingredient.

[0049] Plantain (Plantago major) leaf extract, 1 mg to 1000 mg twice daily, Hit ingredient.

[0050] L-glutathione, 500ug to 1000 mg twice daily. Supports telomere health as described in Consuelo Borra , Juan M. Esteve, Juan R. Vin a, Juan Sastre, Jose Vin a, and Federico V. Pallardo , Glutathione Regulates Telomerase Activity in 313 Fibroblasts, THE JOURNAL OF BIOLOGICAL CHEMISTRY (2004) Vol. 279, No. 33, Issue of August 13, pp. 34332-34335.

[0051] Velvet bean (Mucana pruriens) extract, 1 mg to 1500 mg twice daily. Hit ingredient.

[0052] Hawthorn (Crataegus pinnatifida) root extract, 1 mg to 700 mg twice daily. Hit ingredient.

[0053] Quercetin, 1 mg to 1500 mg twice daily. Hit ingredient.

[0054] Boswellia (Boswellia serrata) fruit extract, 1 mg to 1500 mg twice daily. Hit ingredient.

[0055] Maca (Lepidium meyenii) root extract, 1 mg to 1000 mg twice daily. Hit ingredient.

[0056] Hawthorn (C. pinnatifida) fruit extract, 1 mg to 1500 mg twice daily. Hit ingredient.

[0057] Resveratrol, 1 mg to 500 mg twice daily. Hit ingredient.

[0058] Harada (Terminalia chebula) fruit extract, 1 mg to 500 mg twice daily. Hit ingredient.

[0059] Shilajit extract, 1 mg to 500 mg twice daily. Also known as silajit, mumijo and momia, used in Ayurveda, the traditional Indian system of medicine. Known in industry, through animal models, to have anti-inflammatory, anti-ulcer, anti-anxiety and anti-stress capabilities, dispels pain and has anti-aging (both mental and physical) effects.

[0060] Chia (Salvia hispanica) seed extract, 1 mg to 750 mg twice daily. Contains Omega 3 fatty acids, known to protect telomeres.

[0061] A particular combination, suitable for administration in capsule form, was formulated for potential commercial distribution. Capsules were approximately 470 mg, with 455 mg of activating ingredients and the balance a combination of vitamins C, E and B12 for stability and general health impacts. The activating ingredients comprised about 50% by weight milk thistle seed extract, about 25% in a combination of horny goat weed extract, grape seed extract, turmeric root extract,

ashwagandha root extract, bacopa leaf extract, N-acetyl-L-cysteine, pomegranate fruit extract, DL-alpha lipoic acid, and Asian ginseng root extract, and 25% in a combination of berberine rhizome extract, bilberry fruit extract, blueberry fruit extract, red raspberry fruit extract, green tea leaf extract, white tea leaf extract, black tea leaf extract, acacia bark extract, plantain leaf extract, L-glutathione, velvet bean

extract, hawthorn root extract, quercetin, boswellia fruit extract, maca root extract, hawthorn fruit extract, resveratrol, harada fruit extract, shillajit extract, and chia seed extract.

[0062] WORKING EXAMPLE

[0063] An individual male, 52 years old, had his average telomere length tested by an independent laboratory, Spectracell Laboratories of Houston Texas. Spectracell starts with nucleated white blood cells taken from whole blood and uses the Quantitative PCR technique described in Cawthon, Telomere Measurement by Quantitative PCR, Nucleic Acids

Research, Vol. 30, No. 10 (2002). Initial testing yielded an average telomere length of 8.3 kb, putting the individual in the 78th percentile based on mean telomere length of his age group population.

[0064] The individual began a regimen of taking capsules of Product B, the formulation described above. Three capsules (465 mg each) in the morning and three in the evening were ingested daily, and the subject was re-tested four months later. Using the same methodology, testing yielded an average telomere length of 8.44 kb, putting the individual in the 83rd percentile.

[0065] The foregoing description has been presented and is intended for the purposes of illustration and description. It is not intended to be exhaustive nor limit the invention to the above teachings. The embodiments were chosen and described in order to best explain the principles of the invention and its practical application and to enable others skilled in the art to best utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. Therefore, it is intended that the invention not be limited to the particular embodiments disclosed for carrying out the invention.