SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
PLANT BIOTECHNOLOGY
BIOLOGICAL TECHNIQUES

Received
22 August 2014

Accepted
24 December 2014

Published
23 January 2015

Correspondence and requests for materials should be addressed to L.H. (huangluqi01@126.com)

* These authors contributed equally to this work.

Analysis of the age of *Panax ginseng* based on telomere length and telomerase activity

Jiabei Liang[1,2]*, Chao Jiang[1,3]*, Huasheng Peng[1], Qinghua Shi[4], Xiang Guo[4], Yuan Yuan[1] & Luqi Huang[1]

[1]State Key Laboratory Breeding Base of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, P.R. China, [2]College of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, People's Republic of China 610072, [3]Beijing Area Major Laboratory of Protection and Utilization of Traditional Chinese Medicine, College of Resources, Beijing Normal University, Beijing, People's Republic of China 100875, [4]State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China.

Ginseng, which is the root of *Panax ginseng* (Araliaceae), has been used in Oriental medicine as a stimulant and dietary supplement for more than 7,000 years. Older ginseng plants are substantially more medically potent, but ginseng age can be simulated using unscrupulous cultivation practices. Telomeres progressively shorten with each cell division until they reach a critical length, at which point cells enter replicative senescence. However, in some cells, telomerase maintains telomere length. In this study, to determine whether telomere length reflects ginseng age and which tissue is best for such an analysis, we examined telomerase activity in the main roots, leaves, stems, secondary roots and seeds of ginseng plants of known age. Telomere length in the main root (approximately 1 cm below the rhizome) was found to be the best indicator of age. Telomeric terminal restriction fragment (TRF) lengths, which are indicators of telomere length, were determined for the main roots of plants of different ages through Southern hybridization analysis. Telomere length was shown to be positively correlated with plant age, and a simple mathematical model was formulated to describe the relationship between telomere length and age for *P. ginseng*.

Ginseng, which is the root of *Panax ginseng* C.A. Meyer (Araliaceae), has been used in Chinese medicine for thousands of years as a stimulant and dietary supplement[1]. In European and American countries, ginseng phytomedicines have been used to increase physical and mental performance, provide resistance to stress and disease, and prevent exhaustion for decades[2]. Ginseng plants begin flowering in their fourth year, and the roots can live for hundreds of years after maturing at 4–6 years of age. The older the root, the higher its medicinal value because of the higher concentration of ginsenosides, which are the active chemical compounds in ginseng[3,4]. However, chemical analyses often require gram quantities of dried ginseng material, and it is difficult to extract such quantities while leaving the ginseng intact; thus, chemical analysis greatly decreases the ginseng's value. Therefore, effective methods for identifying the age of ginseng roots are urgently needed to improve quality control and protect the interests of ginseng consumers.

Telomeres, which are specialized structures at the physical ends of eukaryotic chromosomes that consist of highly conserved, repeated DNA sequences[5,6], shorten with each round of DNA replication[7–10] because DNA polymerases cannot completely replicate linear DNA molecules. In gymnosperms, telomere length can be used to predict the future replicative capacity of cells[11,12]. Highly significant correlations between telomere length and age have been observed in humans[8,13], Australian sea lions[14], martins and dunlins[15] and different stages of barley[16]. Therefore, telomere shortening can be used as a marker of cell replication and aging. Telomerase activity has been detected in plants using a polymerase chain reaction (PCR)-based telomerase repeat amplification protocol (TRAP) assay[17]. Telomerase appears to be developmentally regulated in plants, which is similar to what occurs in humans[18]. These reports indicate biological correlations between telomere length and age. However, plant telomeres are maintained by telomerase. Telomere lengths remain stable in tomato leaves[19], whereas they change cyclically, lengthening and shortening with age, in the needles of *Pinus longaeva*[20]. After the first plant telomere sequence was cloned from *Arabidopsis*[21], nearly all plant telomeres were found to consist of the heptanucleotide

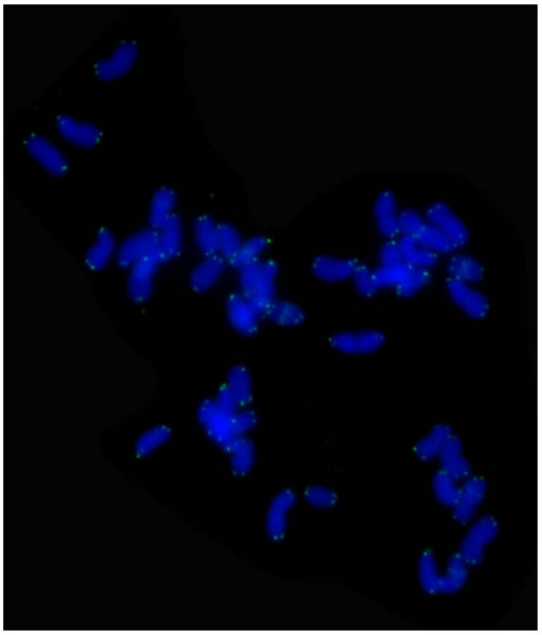

Figure 1 | *In Situ* Localization of TTTAGGG Telomeric Motifs on *P. ginseng* Chromosomes. The the digoxigenin–dUTP nick tag sequence $(CCCTAAA)_5$ telomeric probe was hybridized with adventitious root of *P. ginseng* metaphase chromosomes and counterstained with propidium iodide.

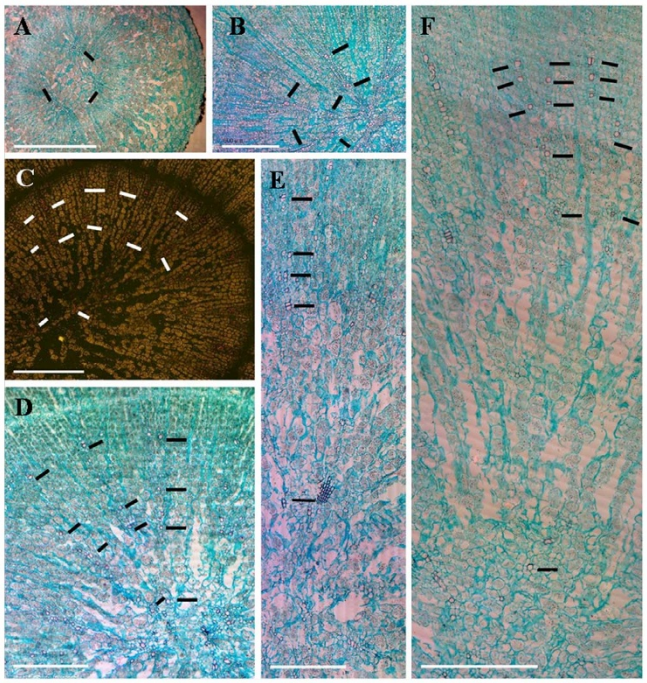

Figure 2 | The growth rings in the ginseng root of 1 ~ 6 years. (A), (B), (C): 1 year ginseng root, 2 year ginseng root, 3 year ginseng root, Bar = 1000 μm; (D), (E), (F): 4 year ginseng root, 5 year ginseng root, 6 year ginseng root, Bar = 500 μm.

repeat (TTTAGGG)n[22,23]. *Arabidopsis*-type repeats have also been found in *P. ginseng*[24]. However, researchers have not yet ascertained whether *Arabidopsis*-type repeats are located in telomeres or their relationship with age.

In this study, we combined traditional identification methods and measurements of telomere length in ginseng plants of known age. Preliminary investigations indicated that telomere length was slightly positively correlated with the age of the ginseng plant. Analysis of telomerase activity in different parts of the plant further revealed that the main root was the most active meristematic region. Therefore, we used this tissue to evaluate telomere length. Determination of telomere terminal restriction fragment (TRF) lengths in *P. ginseng* specimens of different ages demonstrated that the telomeres in the main roots showed a significant increase in TRF length with plant age that could be used for age estimation for 2–8 years.

Results

Fluorescence *in situ* hybridization to determine telomere sequences. The telomeres of most higher plant species are composed of the repeated sequence (TTTAGGG)n. To investigate *P. ginseng* telomeres comprising the same repeat, we using the complementary end digoxigenin-labeled, telomere-specific oligonucleotide $(CCCTAAA)_3$ as a probe to perform *in situ* hybridization. Hybridization signals visualized as green fluorescence demonstrated that *Arabidopsis*-type telomeric sequence repeats, $(TTTAGGG)_n$, were located in the chromosomes of *P. ginseng* (Fig. 1).

Growth rings in the roots of *P. ginseng* from Ji'an. The paraffin sections of *P. ginseng* rhizomes of different ages collected from Ji'an revealed distinct growth rings in the xylem of secondary roots, and the number of growth rings in the main root was consistent with an age of 1–6 years (Fig. 2). However, microscopy analysis showed that the growth rings of the ginseng specimens did not precisely reflect age after 6 years.

Telomeric activity of different ginseng tissues. A representative TRAP analysis image that was used to quantify telomerase activity is shown in Fig. 3. Average telomerase activities in various tissues and different stages of plant development were assayed using TRAP, and the results indicated that the main root showed the highest average telomerase activities of all of the examined tissues. Because telomerase can lengthen telomeres, and the activity of telomerase

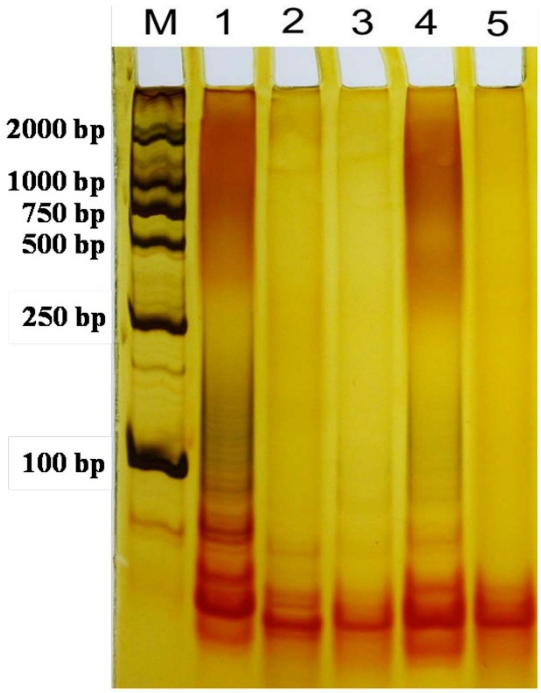

Figure 3 | Developmental Regulation of Telomerase Expression in 5 years *P. ginseng*. Telomerase activities in various tissues and different stages of plant development were assayed by TRAP, using 47F as the forward primer and PTelC3 as reverse primers. Lane1: tap root; Lane2: leaves; Lane3: stems; Lane4: root tips; Lane5: seeds.

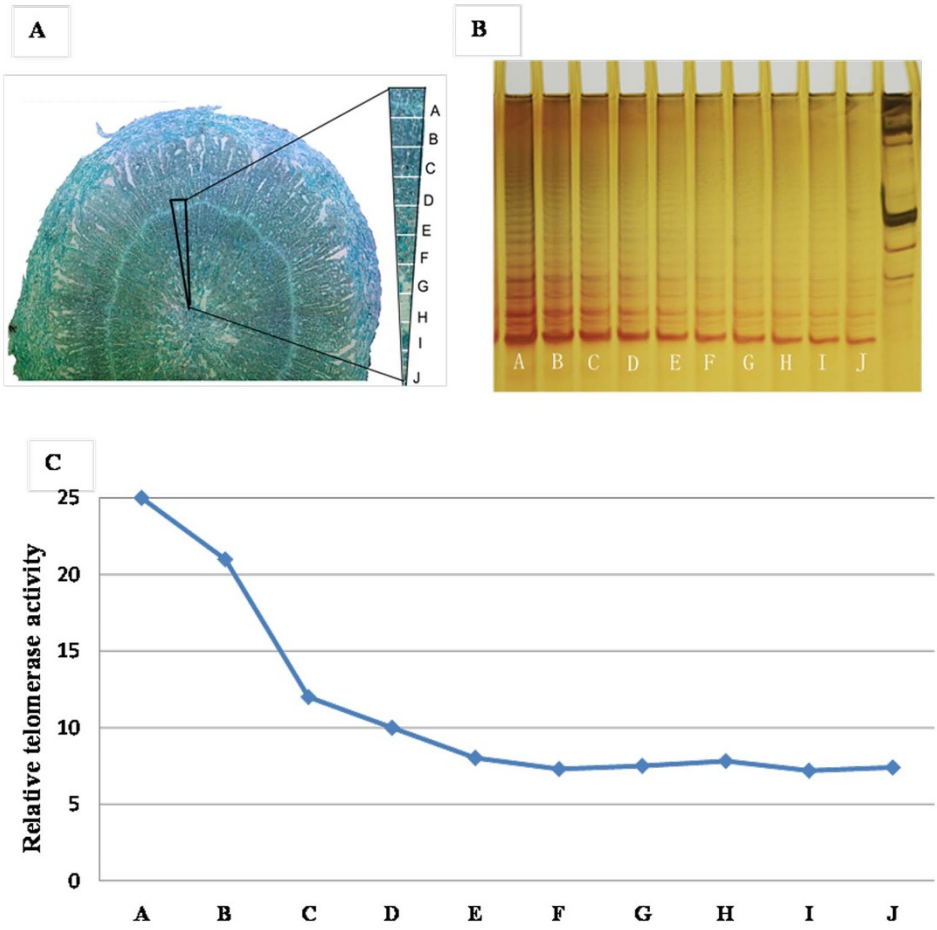

Figure 4 | Anatomical observation of 5 years *P. ginseng* by tangential cryosectioning. Aseries of 400-um-thick tangential cryosections (A) ~ (J) was taken for each sample: tissues at different stages were isolated by tangential cryosectioning; (B): Telomerase activities in various tissues and different stages of plant development were assayed by TRAP, using 47F as the forward primer and PTelC3 as reverse primers; (C): Densitometric quantization revealed higher relative telomerase activity (relative units) in cambiums.

may be correlated with age, the main roots were used for further analyses.

Tangential cryo-sectioning of 400 μm sections of samples from 5-year-old *P. ginseng* tissues, followed by densitometric quantitation of telomerase activity (in relative units), revealed the highest telomerase activity in the cambium and adjacent zones of differentiating secondary xylem (Fig. 4).

Analysis of TRF lengths in ginseng of different ages. DNA fragments were analyzed through DNA gel blot hybridization using the $(CCCTAAA)_3$ oligonucleotide as a probe. A representative Southern blot image that was used to quantify TRFs is shown in Fig. 5a, b, where the hybridization signals represent telomeric regions. The autoradiograph was scanned and imported as a TIFF-format image to measure TRF length. The location of the peak intensity could not be accurately determined by eye. Therefore, an easy-to-use system that was able to determine the distributions of telomeric regions based on copy number and calculate statistics was employed. The unbiased TRF measure software Telotool[25] was used to measure the TRF lengths of ginseng roots. A plot of the relative telomere copy number versus molecular weight was created, which provided the user with a realistic picture of the actual distribution of telomeric lengths. The measurements of TRF length for each sample using Southern hybridization was repeated three times.

We investigated the correlation between TRF length and plant age using *P. ginseng* samples of known age from Ji'an and Fusong. First, DNA fragments were analyzed through Southern hybridization using the $(CCCTAAA)_3$ oligonucleotide as a probe for telomeric DNA (Fig. 5a). Although observations made by eye are not precisely accurate, this easy-to-use method is convenient and allowed rapid analysis of the telomeres of ginseng roots by determining copy number[26]. A general model for age-related TRF length in ginseng was introduced. Eleven models were simulated using SPSS 20.0 software, and the most suitable linear fitting curve was determined. The obtained results satisfied the 83% confidence limits ($R^2 = 0.832$, $F = 79.029$, $Sig. = 0.000$), and it was found that TRF length was significantly positively correlated with age after 3 years (Fig. 5b), which indicated that TRF length could be maintained via telomerase activity as tissues developed. Based on these results, we propose a mathematical model through which telomere length can be used to predict *P. ginseng* age:

$$y = 0.827x + 8.231,$$

where, x is age, and y is TRF length.

Discussion

Scientific identification of the potency of traditional Chinese medicines is crucial to ensure their authenticity and effectiveness. Authenticity can be assured based on several factors: the geographic origin or cultivation source of the species; proper harvesting and processing methods; and growth stage[27]. These factors are all important for the quality of Chinese herbal medicines. Because bioactive secondary compounds accumulate as medicinal plants such as *P. ginseng*, *Salvia miltiorrhiza* and *Coptis chinensis* age, older plants

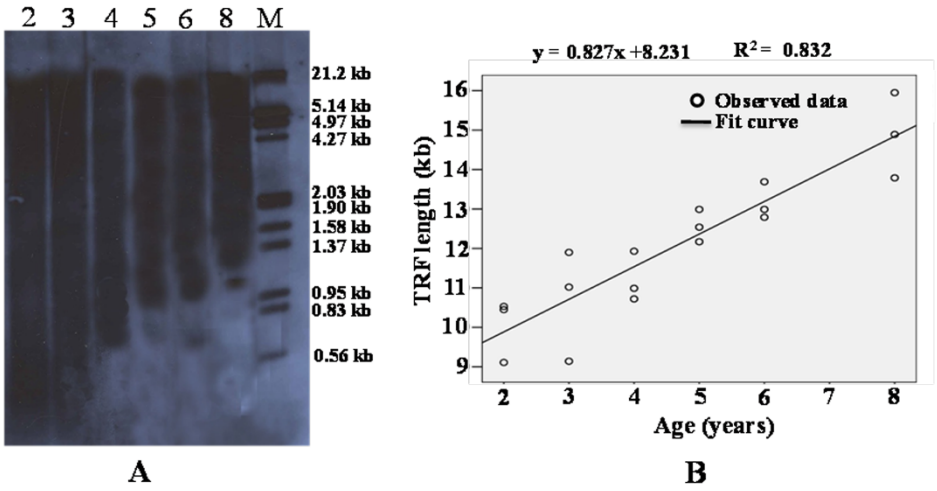

Figure 5 | Southern hybridization images used for measurement and quantization of TRF length. Lane M: DNA Molecular Weight Marker III, Digoxigenin-labeled (Roche). Numbers 2, 3, 4, 5, 6, 8 means different years of *P. gensing* samples collected from the city of Ji'an, Jilin province, China. B: Data fitting results and the trend of variation of TRF length with different ages. Overall, average TRF length increased with ages in main root (The following 1 cm of "ginseng lutou").

usually serve as better medicinal herbs. However, in the pursuit of economic efficiency, a number of inappropriate strategies, including the use of growth hormones and swelling agents as well as continual transplantation, have been employed to simulate age. Therefore, the quality of Chinese herbal medicines is difficult to determine. This study aimed to establish a reliable and effective method for identifying the age of ginseng that complements traditional methods of age determination.

Gymnosperms and dicotyledonous angiosperms generally undergo primary and secondary growth, whereas monocots usually lack secondary growth. The retention of stem-cell-like meristematic cells plays a critical role in perennial longevity[28]. Stem-cell-like meristematic cells are located in the cambium. Accordingly, when environmental conditions change periodically, associated with different growing seasons, the cambium cell cycle is activated, and the tissue layers form rings (termed growth rings) during each individual period of growth. Arx, Schweingruber and Dietz[29,30] indicated that growth rings could be an effective biomarker for estimating age in the roots of dicotyledonous perennial herbs. In the present study, growth-ring characteristics were clearly present in 1- to 6-year-old ginseng top roots. However, when the ginseng specimens were older than 6 years, dry, decayed channels emerged within the cambium rings, making the growth rings difficult to distinguish and influence age estimation. Therefore, this method can only be applied over a minimum age range, and a new marker was required for estimation of the age of older ginseng samples.

Telomere length and telomerase activity are useful biomarkers for age prediction in animals and plants[31–33] due to their close association with cell proliferation. However, it was unclear whether telomerase activity is related to the mechanisms maintaining stem cells in meristems. Our analyses of several *P. ginseng* tissues showed that telomerase activity was highest in the cambium. Telomerase expression in plants is very similar to that in humans. In plants, telomerase activity is highest in the meristem and reproductive organs, whereas there is little or no activity in the endosperm, leaves and stems[17]. In *Ginkgo biloba*, tissues with a high percentage of dividing cells also exhibit high levels of telomerase activity, which is consistent with our results[31,34]. We found that the sampled tissue had a substantial impact on the age estimation in *P. ginseng*. The main root samples contained most of the organized cambium and annual growth rings. We found that telomere length in the main roots was positively correlated with plant age. However, due to sampling limitations, ginseng plants of older ages were difficult to sample. Therefore, our mathematical model is only suitable for a certain range of ginseng ages.

A study that examined TRF branch length in detail suggested that telomere branch lengths increase with age to some extent in *G. biloba*[31,35], in accord with the results of the present study. Our analyses indicate that ginseng telomere length increases significantly with age; however, in contrast to the progressive shortening of TRFs observed in somatic cells as animals aging, telomere length and telomerase activity change in different patterns during plant development. Telomere lengths have been observed to be stable in tomato leaves in four-week-old to six-month-old plants[19], and they do not significantly change during plant ontogenesis or leaf senescence in *Melandrium album*[18] and *Arabidopsis thaliana*[36] or during cyclical changes of lengthening and shortening in size associated with age in *Pinus longaeva*[20]. Furthermore, telomeres do not shorten during increased tissue differentiation from embryonic to adult stages in *Hordeum vulgare*[16] and *Pinus sylvestris*[37], whereas they show decreased lengths during *Betula pendula* tissue culture[38], while increased lengths are observed with age in *G. biloba*[31]. These results suggest that the relationship between telomere length and plant development is complex and may be affected by the species and lines involved as well as environmental stress and telomerase and stem cell activities. The present study indicates that telomere length in the top roots of *P. ginseng* increases with age, as observed in the leaves and calli of *G. biloba*[31,35]. Interestingly, many studies show that ginsenoside Rg1, which is one of the main biologically active components of ginseng, can decrease telomere shortening and reinforce telomerase activity in delayed hematopoietic stem cells and reduce senescence in human somatic cells[39–41]. Similar results were found for a G. *biloba* extract, which significantly augmented endothelial progenitor cell telomerase activity to prevent the cells from entering senescence[42]. These results imply that the increase in telomere length with age

Table 1 | The Ginseng samples collected from two different districts

Age (year)	2	3	4	5	6	8
Fusong	√	√	√	√	√	
Ji'an	√	√	√	√	√	√

Ginseng samples of known age were collected from the Ji'an and Fusong districts of Jilin Province, China, in mid-August, 2010 and 2013. The samples were taxonomically identified by Prof. Shiquan Xu, Institute of special animal and plant science, Chinese Academy of Agricultural Sciences. All samples consist of 3 individuals every year.

observed in ginseng and ginkgo may be related to the bioactive components of these plants, which may maintain telomere length by the telomerase mechanism or/and the ALT mechanism. The correlation between telomere length and telomerase activity in *P. ginseng* that was demonstrated here suggests that telomere length and telomerase activity might play essential roles in directly or indirectly regulating the life span of *P. ginseng*.

Methods

Sample collection. Ginseng samples of known age were collected from the Ji'an and Fusong districts of Jilin Province, China, in mid-August, 2010 and 2013 (Table 1). Samples of the main root (1 cm below the rhizome, known as "ginseng lutou" in China), leaf, stem, secondary root and seeds were frozen in liquid nitrogen and stored at −80°C until use.

Chromosome preparation and *in situ* hybridization. Adventitious roots was induced from calli of *P. ginseng* by cultured in the MS rooting medium for 14 d. Adventitious root tips were used as a source of metaphase chromosomes, and the digoxigenin–dUTP nick (Roche, Penzberg, Germany) tag sequence (CCCTAAA)5 was used as a chromosome probe, as previously described[43]. Slides were removed immediately after immersion in 1 × PBS (containing 0.2% Tween) and dipped in 1 × blocking buffer (Boehringer, Ingelheim, Germany) at 37°C for 30 min. After drying, each slide was placed in 50 × blocking buffer containing 2 μl of a FITC-conjugated anti-digoxin antibody (anti-Dig FITC, Boehringer, Ingelheim, Germany), covered with a 22 × 22 mm coverslip, and incubated in a dark, wet box at 37°C for 60 min. The slides were then washed three times (5 min each) in 1 × PBS (containing 0.2% Tween) at room temperature. After the slides were dried, 12 μl of the anti-fading agent VECTASHIELD® (Vector Lab, California, USA) containing 1 μg/ml propidium iodide (Life Technologies, California, USA) was added slowly to cover the coverslips. Different filters in a DMRXA fluorescence microscope (Leica Microsystems, Wetzlar, Germany) were then used to observe the red chromosomes and yellow-green hybridization signals. An air-cooled digital (CCD) camera was employed, and the input of images into a computer was performed using Leica QFISH software to adjust the contrast and brightness.

Paraffin sectioning. Fresh roots of *P. ginseng* were harvested, and samples were collected 1 cm from the root tip and fixed with FAA solution (70% 90 : 5 : 5 ethanol: formaldehyde: acetic acid), then vacuum infiltrated and dehydrated through increasing alcohol concentrations. Next, the sections were embedded in paraffin, and the preparations were baked on an HI1220 flattening table and sectioned using an RM2265 rotary microtome (Leica Biosystems, Nussloch, Germany) to a thickness of 10–15 μm. The samples were subsequently baked for more than 24 h, then deparaffinized, stained with a safranin–fast green or phloroglucinol–HCl reagent, mounted with neutral gum and observed and photographed using BH-2 optical and LG-PS2 stereo microscopes (Olympus, Tokyo, Japan).

Hand sectioning. One-centimeter samples from the tips of fresh *P. ginseng* roots were sectioned at a thickness of approximately 1 mm. One drop of phloroglucinol-HCl was used to develop color, and a scanner was employed to image the samples.

Tangential cryo-sectioning. A series of 40 μm-thick tangential sections were obtained for each sampled section as described by Uggla & Sundberg[44], with some modifications. Regenerated tissues at different stages were isolated through tangential cryo-sectioning at −20°C with a Leica CM1850 Cryostat (Leica Biosystems, Nussloch, Germany). Cryosections of regenerated tissues from the same root and stage were collected in a 1.5 ml microfuge tubes, immediately frozen in liquid nitrogen and stored at −70°C.

Determination of telomerase activity. Total protein was harvested from approximately 5.0 mg of freshly ground, fine powder from each sample. Telomerase activity was measured as previously described[45] using the TRAP assay. Plant extracts containing telomerase were prepared according to Fitzgerald[17], and the total protein content in the extracts was determined[46]. The oligonucleotides 47F (5′-CGCGGTAGTGATGTGGTTGTGTT-3′) and PTelC3 (5′-CCCTAAACCCTAAACCCTAAA-3′) were used as forward and reverse primers, respectively, in the TRAP analysis[18].

DNA extraction and Southern hybridization analysis. The TRF length, which is the gold standard for telomere length, was determined through Southern hybridization analysis[47]. Root samples were placed in a mortar and ground to a fine powder using a pestle and liquid nitrogen. Then, genomic DNA was prepared from each sample using the hot CTAB method[48] and subsequently purified by treated with RNase (New England Biolabs, Massachusetts, USA) and Proteinase K (Merck, Darmstadt, Germany). The concentration of the isolated DNA and the ratio of the absorbance at 260 nm to 280 nm (A_{260}/A_{280} ratio) were measured using a NanoDrop ND-1000 spectrophotometer (Gene, Hong Kong, China). Approximately 20 μg of each DNA sample was then digested for 12 h with Taq^{a}I, and the digestion products were loaded into the lanes of a horizontal, 6.5 × 10 cm, 1% agarose gel and electrophoresed in 1 × TAE buffer for approximately 6 h at 90 V at room temperature with buffer recirculation. To measure and quantify the TRFs, Southern hybridizations were performed with the DIG High PrimeDNA Labeling and Detection Starter kit II (Roche, Penzberg, Germany) as previously described[49,50] using an end digoxigenin-labeled complementary telomere-specific oligonucleotide probe $(CCCTAAA)_3$. Measurements were repeated three times, and TRF lengths are reported as the mean ± standard deviation.

1. Patil, D. A. Ethnomedicine to modern medicine: genesis through ages. *J. Exp. Sci.* **2**, 25–29 (2011).
2. Gillis, C. N. *Panax ginseng* pharmacology: a nitric oxide link? *Biochem. Pharmacol.* **54**, 1–8 (1997).
3. Chen, F., Luo, J. & Kong, L. Determination of 10 ginsenosides in *Panax ginseng* of different harvest times based on HPLC fingerprints and principal component analysis. *Nat. Prod. Res.* **27**, 851–854 (2013).
4. Kim, N. *et al.* Nontargeted metabolomics approach for age differentiation and structure interpretation of age-dependent key constituents in hairy roots of *panax ginseng*. *J. Nat. Prod.* **75**, 1777–1784 (2012).
5. Azanza, R. V. & Taylor, F. J. Are Pyrodinium blooms in the Southeast Asian region recurring and spreading? A view at the end of the millennium. *Ambio.* **30**, 356–364 (2001).
6. Kipling, D., Wilson, H. E., Thomson, E. J. & Cooke, H. J. YAC cloning Mus musculus telomeric DNA: physical, genetic, in situ and STS markers for the distal telomere of chromosome 10. *Hum. Mol. Genet.* **4**, 1007–1014 (1995).
7. Olovnikov, A. M. Telomeres, telomerase, and aging: origin of the theory. *Exp. Gerontol.* **31**, 443–448 (1996).
8. Harley, C. B., Futcher, A. B. & Greider, C. W. Telomeres shorten during ageing of human fibroblasts. *Nature.* **345**, 458–460 (1990).
9. Olovnikov, A. M. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J. Theor. Biol.* **41**, 181–190 (1973).
10. Watson, J. D. Origin of concatemeric T7 DNA. *Nat. New Biol.* **239**, 197–201 (1972).
11. Murray, B. G., Friesen, N. & Heslop-Harrison, J. S. Molecular cytogenetic analysis of Podocarpus and comparison with other gymnosperm species. *Ann. Bot.* **89**, 483–489 (2002).
12. Allsopp, R. C. *et al.* Telomere length predicts replicative capacity of human fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* **89**, 10114–10118 (1992).
13. Hastie, N. D. *et al.* Telomere reduction in human colorectal carcinoma and with ageing. *Nature.* **346**, 866–868 (1990).
14. Izzo, C., Hamer, D. J., Bertozzi, T., Donnellan, S. C. & Gillanders, B. M. Telomere length and age in pinnipeds: The endangered Australian sea lion as a case study. *Mar. Mammal Sci.* **27**, 841–851 (2011).
15. Pauliny, A., Wagner, R. H., Augustin, J., Szép, T. & Blomqvist, D. Age-independent telomere length predicts fitness in two bird Species. *Mol. Ecol.* **15**, 1681–1687 (2006).
16. Kilian, A., Stiff, C. & Kleinhofs, A. Barley telomeres shorten during differentiation but grow in callus culture. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 9555–9559 (1995).
17. Fitzgerald, M. S., McKnight, T. D. & Shippen, D. E. Characterization and developmental patterns of telomerase expression in plants. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 14422–14427 (1996).
18. Riha, K., Fajkus, J., Siroky, J. & Vyskot, B. Developmental control of telomere lengths and telomerase activity in plants. *Plant Cell.* **10**, 1691–1698 (1998).
19. Broun, P., Ganal, M. W. & Tanksley, S. D. Telomeric arrays display high levels of heritable polymorphism among closely related plant varieties. *Proc. Natl. Acad. Sci. U. S. A.* **89**, 1354–1357 (1992).
20. Flanary, B. E. & Kletetschka, G. Analysis of telomere length and telomerase activity in tree species of various life-spans, and with age in the bristlecone pine *Pinus longaeva*. *Biogerontology.* **6**, 101–111 (2005).
21. Richards, E. J. & Ausubel, F. M. Isolation of a higher eukaryotic telomere from Arabidopsis thaliana. *Cell.* **53**, 127–136 (1988).
22. Ganal, M. W., Lapitan, N. L. & Tanksley, S. D. Macrostructure of the tomato telomeres. *Plant. Cell* **3**, 87–94 (1991).
23. McKnight, T. D., Fitzgerald, M. S. & Shippen, D. E. Plant telomeres and telomerases. A review. *Biochemistry.* **62**, 1224–1231 (1997).
24. Ho, I. S. & Leung, F. C. Isolation and characterization of repetitive DNA sequences from *Panax ginseng*. *Mol. Genet. Genomics.* **266**, 951–961 (2002).
25. Göhring, J., Fulcher, N., Jacak, J. & Riha, K. TeloTool: a new tool for telomere length measurement from terminal restriction fragment analysis with improved probe intensity correction. *Nucleic. Acids. Res.* **42**, e21 (2014).
26. Grant, J. D. *et al.* Telometric: a tool providing simplified, reproducible measurements of telomeric DNA from constant field agarose gels. *Biotechniques.* **31**, 1314–1316, 1318 (2001).
27. Huang, L., Xiao, P., Guo, L. & Gao, W. Molecular pharmacognosy. *Sci. China Life Sci.* **53**, 643–652 (2010).
28. Lanner, R. M. Why do trees live so long? *Ageing Res. Rev.* **1**, 653–671 (2002).
29. Arx, G. & Dietz, H. Growth rings in the roots of temperate forbs are robust annual markers. *Plant Biol.* **8**, 224–233 (2006).
30. Dietz, H. & Schweingruber, F. H. Development of growth rings in roots of dicotyledonous perennial herbs: experimental analysis of ecological factors. *Bull. Geobot. Inst. ETH.* **67**, 97–105 (2001).

31. Liu, D. *et al*. Comparative analysis of telomeric restriction fragment lengths in different tissues of *Ginkgo biloba* trees of different age. *J. Plant Res.* **120**, 523–528 (2007).
32. Mather, K. A., Jorm, A. F., Parslow, R. A. & Christensen, H. Is telomere length a biomarker of aging? A review. *J. Gerontol. A.* **66**, 202–213 (2011).
33. Watson, J. & Riha, K. Telomeres, aging, and plants: from weeds to Methuselah–a mini-review. *Gerontology.* **57**, 129–136 (2010).
34. Song, H., Liu, D., Li, F. & Lu, H. Season-and age-associated telomerase activity in *Ginkgo biloba* L. *Mol. Biol. Rep.* **38**, 1799–1805 (2011).
35. Liu, D. *et al*. Dynamic changes of telomeric restriction fragment (TRF) lengths in cells during the developmental process from embryos to seedlings and a comparison with the embryonal calli in *Ginkgo biloba* L. *Forest. Stud. China.* **9**, 127–131 (2007).
36. Zentgraf, U., Hinderhofer, K. & Kolb, D. Specific association of a small protein with the telomeric DNA-protein complex during the onset of leaf senescence in *Arabidopsis thaliana*. *Plant Mol. Biol.* **42**, 429–438 (2000).
37. Aronen, T. & Ryynänen, L. Variation in telomeric repeats of Scots pine (*Pinus sylvestris* L.). *Tree Genet. Genomes.* **8**, 267–275 (2012).
38. Aronen, T. & Ryynänen, L. Silver birch telomeres shorten in tissue culture. *Tree Genet. Genomes.* **10**, 67–74 (2014).
39. Zhou, B. *et al*. Ginsenoside Rg1 protects human fibroblasts against psoralen-and UVA-induced premature senescence through a telomeric mechanism. *Arch. Dermatol. Res.* **304**, 223–228 (2012).
40. Zhou, Y. *et al*. Changes of telomere and telomerase in effect of ginsenoside Rg1 to delay hematopoietic stem cell senescence. *J. Chin. Mater. Med.* **36**, 3172–3175 (2011).
41. Zhao, C. *et al*. Roles of telomere and telomerase in the process of ginseno-side Rg1 protection against tert-butyl hydroperoxide-induced senescence in WI-38 cells [J]. *Chin. Pharmacol. Bull.* **1**, 61–66 (2005).
42. Dong, X. X. *et al*. Ginkgo biloba extract reduces endothelial progenitor-cell senescence through augmentation of telomerase activity. *J. Cardiovasc. Pharm.* **49**, 111–115 (2007).
43. Kurata, N. & Omura, T. Karyotype analysis in rice, 1: A new method for identifying all chromosome pairs. *Japan. J. Genet.* **53**, 251–255 (1978).
44. Chaffey, N. J. *Wood formation in trees: cell and molecular biology techniques* (CRC Press, Florida, 2004).
45. Kim, N. W. *et al*. Specific association of human telomerase activity with immortal cells and cancer. *Science.* **266**, 2011–2015 (1994).
46. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254 (1976).
47. Nakamura, K. *et al*. Comparative analysis of telomere lengths and erosion with age in human epidermis and lingual epithelium. *J. Invest. Dermatol.* **119**, 1014–1019 (2002).
48. Rogers, S. O. & Bendich, A. J. *Plant Molecular Biology Manual* 73–83 (Springer Press, Heidelberg, Germany, 1989).
49. Flanary, B. E. & Streit, W. J. Telomeres shorten with age in rat cerebellum and cortex in vivo. *J. Anti. Aging Med.* **6**, 299–308 (2003).
50. Flanary, B. E. & Streit, W. J. Progressive telomere shortening occurs in cultured rat microglia, but not astrocytes. *Glia.* **45**, 75–88 (2004).

Acknowledge

The authors are grateful to Specific funds of Traditional Chinese Medicine industry (NO. 201407003) and Key Projects in the National Science & Technology Pillar Program (NO. 2012BAI29B02).

Author contributions

J.L. conducted experiments and data analysis. C.J. conducted data analysis and wrote the paper. H.P. collected the majority of the samples provided the case information and conducted experiments. Q.S. and X.G. conducted the FISH experiments. Y.Y. designed experiments and revised the paper. L.H. designed experiments, performed data analysis and wrote the paper.

Additional information

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Liang, J. *et al*. Analysis of the age of *Panax ginseng* based on telomere length and telomerase activity. *Sci. Rep.* **5**, 7985; DOI:10.1038/srep07985 (2015).