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A natural life expansion enhancer: A new and efficient telomerase booster

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ABSTRACT

It is well known that telomerase is the main agent to protect telomere - the terminal guanine-rich sequences of chromosomes- during cellular division. In order to protect telomeres from shortening we designed a new blend of plant extracts whose part of them are well known since the dawn of Chinese medicine. The length of telomeres was checked with a High Quantification by Fluorescence *In Situ* Hybridization (HQ-FISH) technology so that each cell has been reported with its own telomere parameters. During the first step, experiments were carried out on Medical Research Council. Cell strain 5 (MRC-5, a diploid human cell culture line composed of fibroblasts derived from lung tissue of a 14 week-old aborted Caucasian male fetus) were fed with Dulbecco/Vogt's Modified Eagle's Minimal (DMEM) medium (essential medium contains approximately four times as much of the vitamins and amino acids present in the original formula and two to four times as much glucose. Additionally, it contains iron and phenol red) and a «telomerase activator agent» - coined as TeloBooster®- the compounds of which are protected by a registered patent. During the second step, clinical test was carried out on humans. The results obtained on humans were similar to the laboratory ones: Telomere length can reach a twofold increase more important than the reference group of humans with the same age.

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INTRODUCTION

Plants are used to help keep humans in good health since the dawn of humanity (Smith and Stuart, 1973; Jing, 1998; Dharmananda, 2001; Huang and Wang, 1993) and were used in different ways depending on their effects on the health (Yang, 2002; State Administration of Traditional Chinese Medicine, 1995).

It is well known that telomerase is the main agent to protect telomere during cellular division (Bodnar et al., 1998).

The Nobel Prize of Physiology or Medicine 2009 was awarded to Elizabeth H. Blackburn, Carol W. Greider and Jack W. Szostak for their discovery of the protection of telomeres by the telomerase. Recent tests, in particular on mice, showed the efficiency of certain plants whilst determining that these plants had no carcinogenic effect (Yang, 2002; State Administration of Traditional Chinese Medicine, 1995).

MATERIALS AND METHODS 1

Laboratory experiments

Plant extracts were tested on fibroblast human cells of type MRC-5 and found evidence of a meaningful effect on the extent of human cell life.

Experiments were carried out on a MRC-5 cell strain fed with a DMEM medium (Dulbecco's Modified Eagle's Medium) and the TeloBooster®. The compounds of TeloBooster® are disclosed in the registered patent database (Caterini, 2014). MRC-5 cells are a human diploid fibroblast culture able to duplicate *in vitro* for

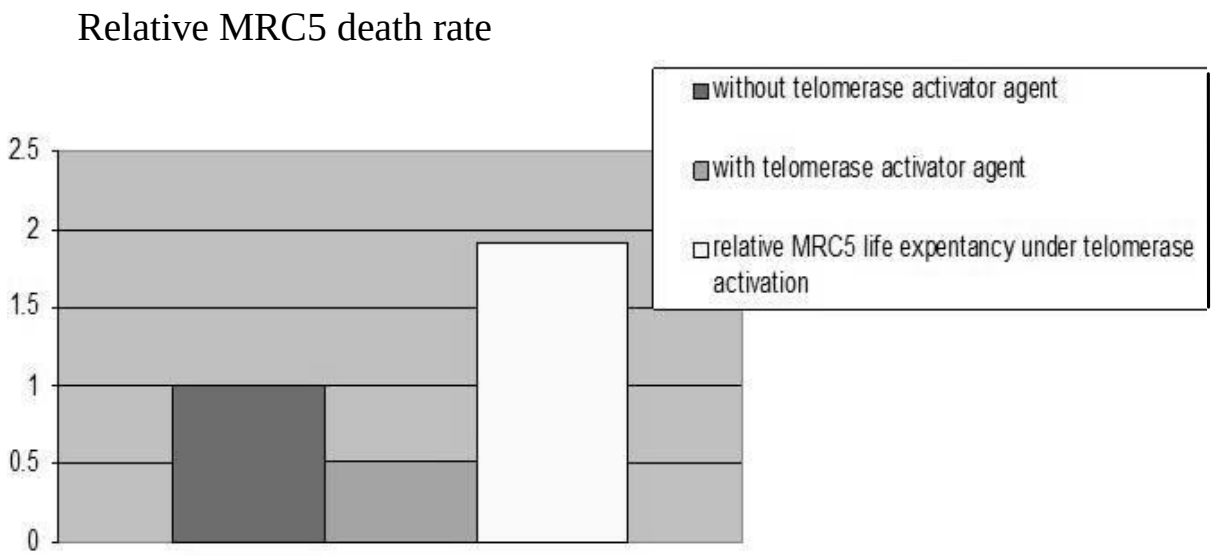

Figure 1. TeloBooster® increases cell life expectancy by a 1.9 times factor (Y-axis).

approximately 50 population doublings, after which they become senescent and cease to replicate i.e. they are a 'finite cell line'.

The strain labeled 11D007, dated 04/2011 and aged 30 population doublings were worked on.

Constituents of medium

The medium is made from DMEM medium and is mixed with these additional compounds:

- Fetal calf serum 10%;
- Glutamine 1%;
- Penicillin (10,000 U/ml) and Streptomycin (10,000 µg/ml) 1%.

The MRC5 sham samples and the MRC-5 fed with TeloBooster® (Caterini, 2014) were grown in wells under usual growing conditions (temperature, pH, osmotic pressure, O_2 and CO_2 tension).

During a first experiment, 6.4% additional cells for MRC-5 fed with TeloBooster® was gotten. After adjusting the percentage of compounds within TeloBooster®, a second experiment was launched and an additional cell increase of 19% was achieved when fed with TeloBooster®. To determine whether the difference between the amount of additional cells in the sham sample and the modified medium resulted from senescent cells still active rather than young divided cells, a new experiment was engaged upon. This experiment's main characteristic lies in the limited space factor for cells to expand forever. This limit on cell expansion blocks the number of cells to a global extent in such a way that all the samples achieved the same number of cells. The optical density activity level of these samples is in direct proportion to the age of MRC-5 cells since cell activity declines as cells age.

To get a global figure on the activity level of our samples, XTT test [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, was worked with; which yields a water-soluble product. This test measures the reduction potential of the cell using a colorimetric reaction].

Samples were grown in two independent series of 12 independent wells for a week.

RESULTS 1

As cells age, the death rate settles as a down slope activity under the XTT test. Given that the death rate of the sample fed without TeloBooster® is settled as «1» a half death rate for experimental samples fed with TeloBooster® was obtained.

Given that the MRC-5 sample fed with TeloBooster® is populated with as many cells as in the MRC-5 sham sample, 2 times more «surviving» cells in TeloBooster® samples was achieved. This led to a conclusion that these surviving cells are «senescent» cells still functioning at an acceptable level of activity according to the XTT test.

As a global conclusion, linked to our previous experiments and data gathered from scientific literature, TeloBooster® helps cells to preserve the telomere caps so that cells could survive a longer time than «natural»

cells usually do (Allsopp et al., 1992). According to our biological model the additional life expectancy under a TeloBooster® prescription is 1.9 times higher than the normal life expectancy (Figure 1).

At the moment such an experimental result was gotten, clinical tests on humans were lunched. These tests were not intended to deploy a large statistical analysis on a group of humans but to check the effect of TeloBooster® on the telomere length of a group of few human subjects.

CLINICAL TRIALS ON HUMANS

These researches are intended to provide a protection to mammalian telomeres so that telomere shortening could decrease in order to increase life expectancy. There is direct evidence for a causal relation between telomere shortening and cellular senescence (Bernardes de Jesus et al., 2012).

There is a consistent shortening of telomeres during cellular aging in culture and with aging of human tissues *in vivo* (Harley et al., 1990). Furthermore, the relation between telomere loss and senescence had been established by finite life-span imposed on mutant yeast cells harboring a defunct telomere maintenance system (Lundblad and Szostak, 1989).

Chromosomes are highly condensed rods of deoxyribonucleic acid (DNA), the genetic material which contains the building blocks of life. DNA carries a specific code that gives instructions to our body on how to grow, develop and function. The instructions are organized into units called genes.

Chromosomes serve as the storage for this important material, periodically dividing along with cells and replicating to make copies of the DNA they contain. Chromosomes are also very important in sexual reproduction, as they allow an organism to pass genetic material on to descendants.

In organisms with cell nuclei, known as eukaryotes, chromosomes are found inside the nucleus. Most of these organisms have a set of chromosomes which come in pairs. In structural cells, each cell retains a complete set of chromosomes, in what is known as diploid form, referring to the fact that the chromosomes contain two copies of each gene.

Telomeres are the ends of chromosomes, which have an essential role in protecting their integrity (Cooke and Smith, 1986). Telomeres are formed by tandem repeats of a DNA sequence, which is conserved throughout evolution (TTAGGG in vertebrates) and associated proteins (the so-called telomere-binding proteins or “shelterins”). The function of telomeres is to protect chromosome ends from DNA repair and degradation activities, therefore, ensuring the proper functionality and viability of cells.

Telomerase is an enzyme which is able to maintain telomeres and repair short telomeres by re-elongating them. To this end, telomerase add telomeric repeats "de novo" to the chromosome ends. In non-pathological conditions, telomerase is expressly associated with pluripotency (early stages of embryo development), as well as in certain adult stem cell compartments. Healthy cells usually produce little or no telomerase and, as a consequence of this, they progressively shorten their telomeres associated to successive cycles of cell division, until they reach a critically short length which triggers cell death or an irreversible cell arrest known as replicative senescence If a cell divides recursively, at some point; all the progeny will reach their Hayflick limit (Hayflick and Moorhead, 1961).

There is strong evidence from genetically modified mouse models that demonstrates that accumulation of critically short telomeres is sufficient to cause organ animal aging and that intervention decreases the rate of telomere shortening with age, such as forced expression of the telomere-synthesizing enzyme (telomerase) is also sufficient to delay aging and increase longevity (Siegel, 2013; Blasco, 2005).

A variety of premature aging syndromes are associated with short telomeres (Chang, 2012). Thus, therapeutic strategies based on telomerase activation are envisioned as potentially important for dealing with age-related problems.

MATERIALS AND METHODS 2

All analyses were led by "Life Length Laboratory" in Spain which is worldwide granted for its technical breakthrough in genetics analysis. Life Length’s technology is the only large scale telomere analysis technology that allows the quantification of the abundance of critically short telomeres. The other high telomere length measurement techniques, such as polymerase chain reaction (PCR) or flow cytometry based methods, can only determine the mean telomere length of a cell or a sample, but are unable to measure the percentage of short telomeres.

Slight changes in the percentage of short telomeres with aging, lifestyle or life quality are not reflected in mean telomere length. Life Length laboratory measures telomere length by telomeric quantitative FISH (Q-FISH) on interphase nuclei both on tissue sections (Telomapping) and, by using High Throughput Quantitative Fluorescence *In Situ* Hybridization (HT Q-FISH technology, where telomeres are hybridized with a telomeric probe marked with fluorescence) on blood cells or any other cell type like fibroblasts, keratinocytes, primary neurons, glial cells, neuroblastomas, and a variety of transfected cell lines that can be attached to a plate.

Each telomeric probe recognizes a fixed number of

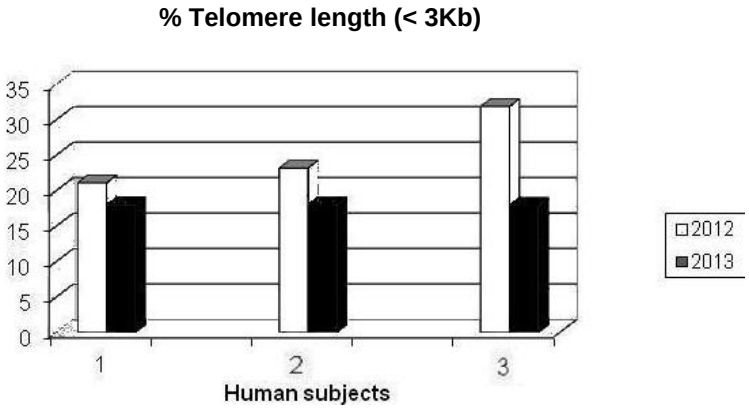

Figure 2. From 2012 to 2013 the laboratory results show that the percentage of short telomeres (< 3 kb) is lower in 2013 than in 2012. It means that TeloBooster® slows down the natural decrease of telomere length. For easier reading there are only 3 subjects displayed in the chart.

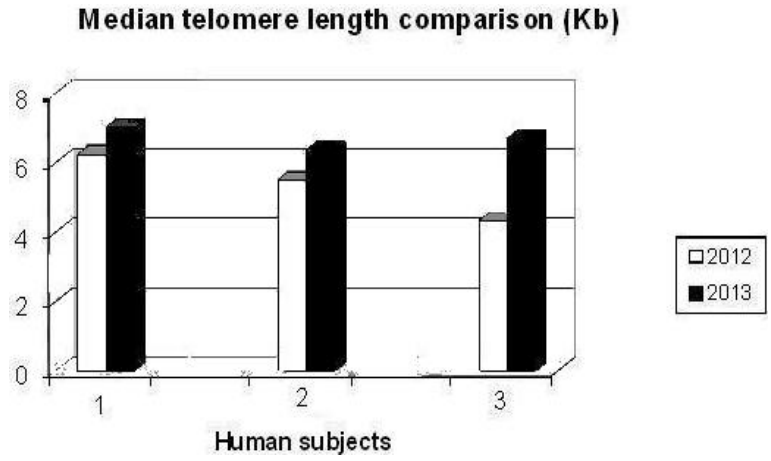

Figure 3. From 2012 to 2013 the laboratory results show that the median telomere length is longer in 2013 than in 2012. It means that TeloBooster® slows down the natural decrease of telomere length. For easier reading there are only 3 subjects displayed in the chart.

telomeric repeats (base pairs). For this reason, the intensity of the fluorescent signal from telomeric probes that hybridize to a given telomere is directly proportional to telomere length. Finally, fluorescence values are transformed into telomere length values for each individual telomere spot within a cell, being able to measure the mean telomere length as well as the percentage of short telomeres in a cell population.

This study is aimed at protecting telomere length from aging and in some extends to maintain their average length so that cells are able to continue to divide and generate healthy and strong management of the body as a whole. On the technical level all subjects underwent a blood sample before the everyday commencement of TeloBooster® for a period of year. The results coming from this first blood sample is labelled as "2012" in the following part of this report (Figures 2 and 3). During a year all human subjects took a capsule of the TeloBooster® every morning. After a year all subjects underwent a second blood sample. The results coming from this second blood sample is labelled as "2013" in the following part of this report (Figures 2 and 3).

RESULTS 2

Raw data are sorted as a histogram showing the distribution and spreading out of cells according to the

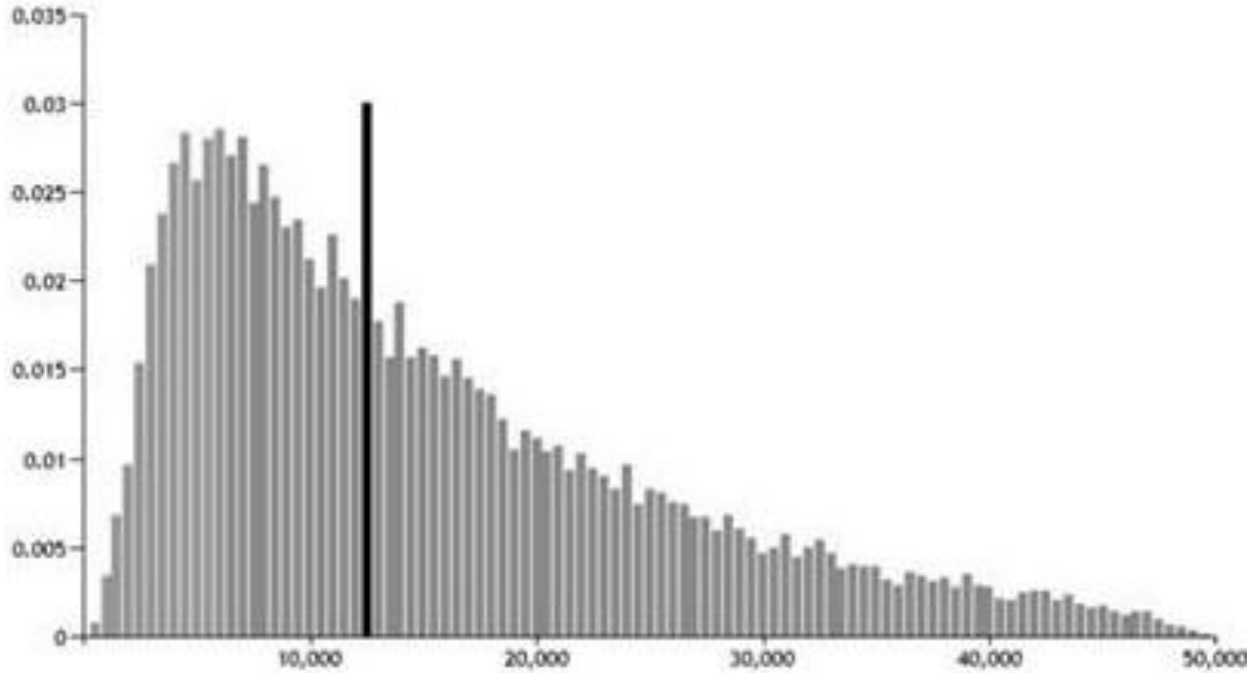

Figure 4. Example of histogram displaying the 20th percentile of the cell population coming from ELSA1001069's subject. The black vertical bar shows the median telomere length. The X-axis displays absolute values of telomere length in Kilo-Bases Pair. Y-axis shows the percentage of cells at the precise telomere length (X-axis). Courtesy of Labco Life Length Laboratories.

length of subjects' telomeres (Figure 4). The 20th percentile indicates the particular length below which 20% of the shortest telomeres have been observed. Therefore, if there are many bars in green, this indicates a relatively low abundance of short telomeres, while if only a few bars are in grey, this indicates a relatively high percentage of short telomeres. The median is also indicated in the histogram and it represents the 50th percentile of the distribution. This histogram also allows for the analysis of telomere length variability. A narrow histogram indicates relative homogeneity in telomere length, while a wider histogram indicates greater telomere length variability which, in turn, could suggest poor telomerase activity and telomere elongation by alternative lengthening mechanisms (that is, recombination).

From these data are extracted all parameters in order to compare each subject to a large database made from a wide population of human subjects. Hence it is possible to localize the biological age of each subject inside of the whole population. According to such a comparison it is possible to compare the biological age for each subject to his/her chronological age.

After a year of an intake of TeloBooster® all results show the same increasing effect in telomere length for all human subjects (Figure 2). As an example of TeloBooster®'s efficiency: The telomeres length of human subject "ELSA001040" grew from 4 to 7 kbp after a year of a daily intake (Figures 5 and 6).

After one year of daily intake of TeloBooster®, the same subject comes into the group of people ranging from 51 to 60 years. These results show that the subject " ESLA001040" belonged to the 70 age group in 2012 but gained several years by the end of 2013. The subject jumped from the 70 aged population to the 60 one in 2013 according to Labco Lifelength Laboratory database (Figure 6).

The protection of telomere length is still working for all human subjects after three years of a regular daily intake of TeloBooster® (Figure 7). After three years all subjects show an additional telomere length that could reach 200% of the initial value.

Conclusion

Although telomere length decreases with aging in everyday life, all human subjects belonging to the study group were granted with a protection of their telomere length under a daily intake of TeloBooster®.

Telomere length extension has successfully reversed some signs of aging in laboratory mice (Jaskelioff et al., 2011). Age-related diseases and premature ageing syndromes are characterized by short telomeres, which

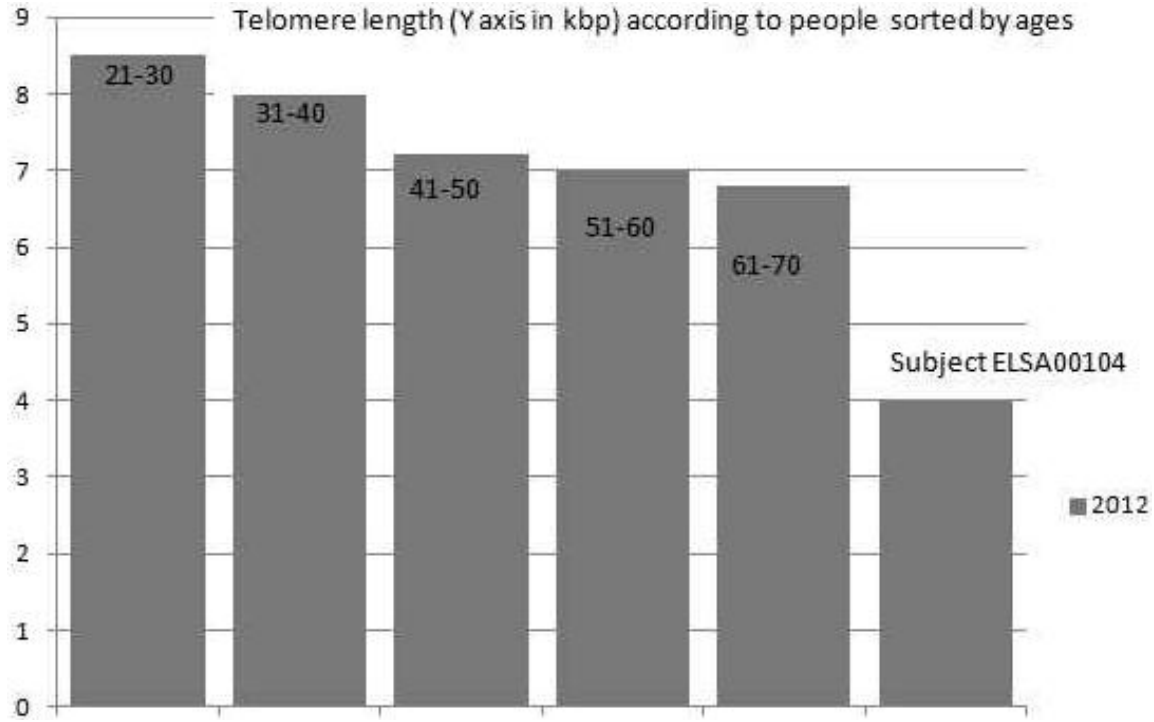

Figure 5. Median telomere length without any daily intake of the TeloBooster® (subject reference ESLA001040). The subject is older than the group of people ranging from 61 to 70 years.

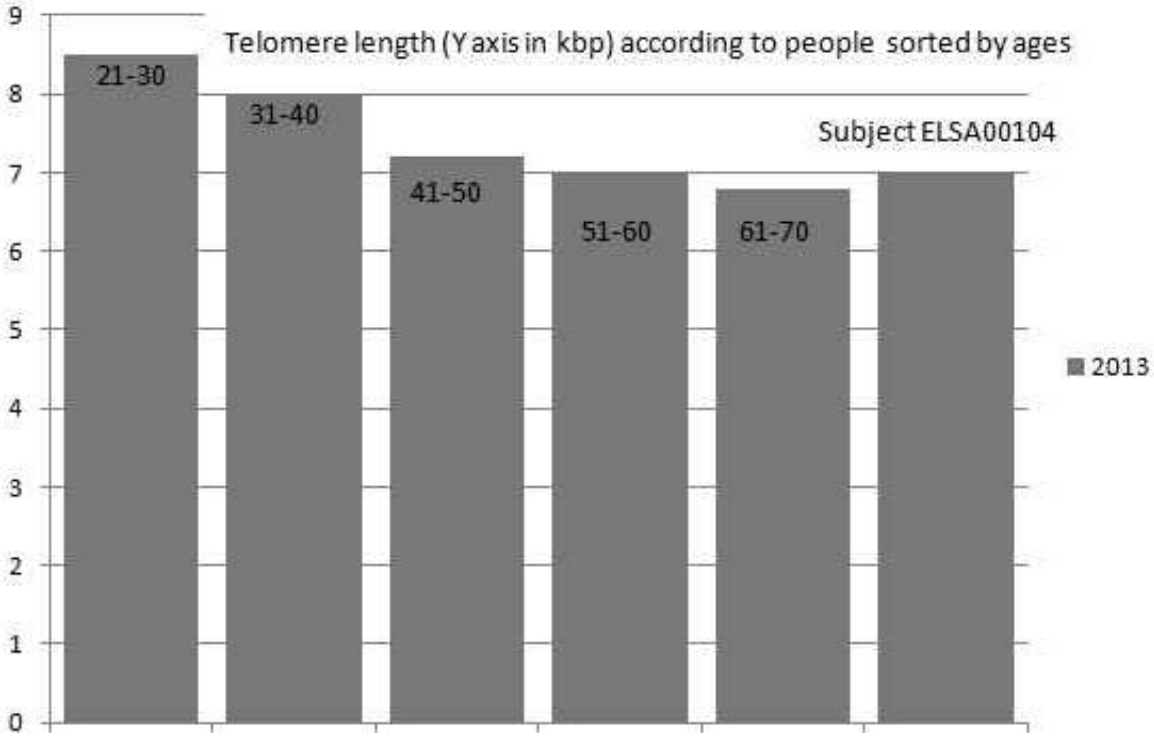

Figure 6. Median telomere length difference after one year of daily intake of TeloBooster® (subject reference ESLA001040).

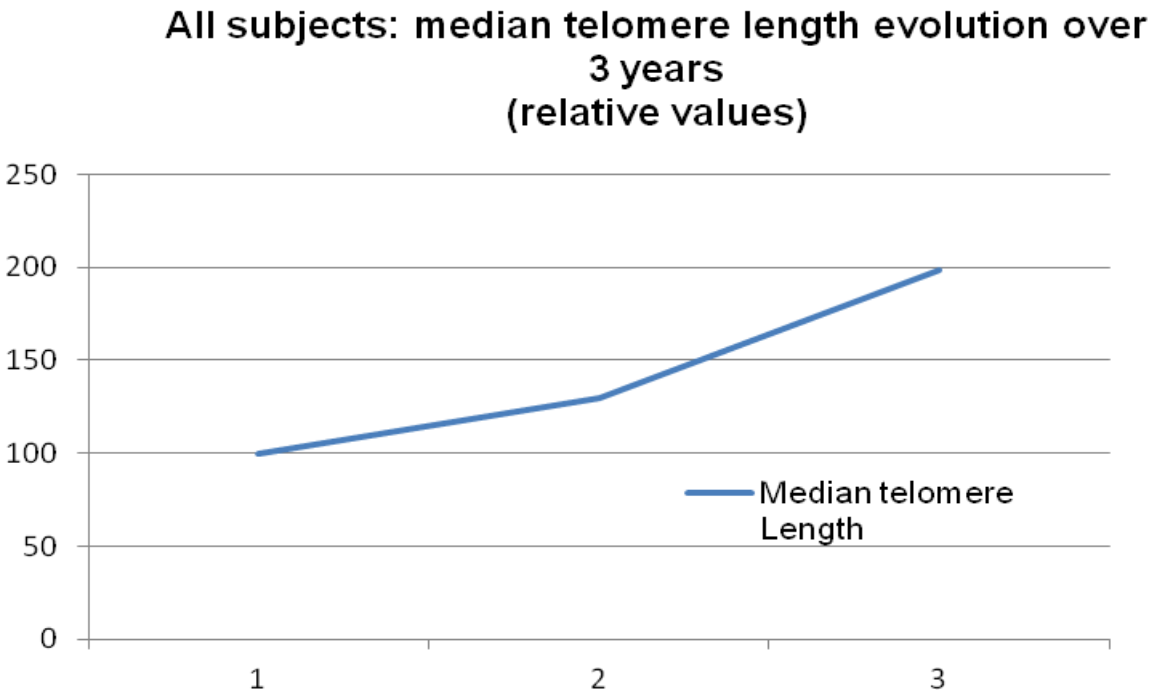

Figure 7. Average increase tendency of the length of telomeres for the group of subjects over three years (Y-axis: Relative average increase of telomere length. X-axis: Years).

can compromise cell viability (Blasco, 2005).

It is well known that telomere length is critical for maintaining genomic integrity and telomere dysfunction or shortening is commonly involved during the process of tumor development (Raynaud et al., 2008; Blasco et al., 1997).

Short telomeres can lead to genomic instability, chromosome loss and the building of non-reciprocal translocations and telomeres in tumor cells are significantly shorter than surrounding normal tissue (Artandi et al., 2000).

Observation studies have found short telomeres in many cancers and people with many types of cancer have shorter leukocyte telomeres than healthy control subjects (Willeit et al., 2010). Recent studies show 1.4 to 3.0 fold increased risk of cancer for people with the shortest vs. longest telomeres (Wentzensen et al., 2011).

It is important to note that recent studies have correlated the likelihood of developing certain types of cancer and the presence of short telomeres in humans (Blasco, 2005). The increase in telomere length, according to this research, reduces the appearance of certain types of cancer (Chang, 2012; Willeit et al., 2010).

According to scientific literature (Bodnar et al., 1998), it means that all human subjects belonging to this experimental group expand their expectancy of life with an intake of our natural product after only a year of treatment.

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