Food Supplement 20070721-GX May Increase CD34\(^+\) Stem Cells and Telomerase Activity

Po-Cheng Lin,\(^1\) Tzyy-Wen Chiou,\(^2\) Po-Yen Liu,\(^3\) Shee-Ping Chen,\(^4\) Hsin-I Wang,\(^5\)
Pi-Chun Huang,\(^5\) Shinn-Zong Lin,\(^1,6,7\) and Horng-Jyh Harn\(^8,9\)

\(^1\) Center for Neuropsychiatry and Department of Neurosurgery, China Medical University Hospital, 2 Yuh-Der Road, Taichung 40447, Taiwan
\(^2\) Department of Life Science and Graduate Institute of Biotechnology, National Dong Hwa University, Hualien 97401, Taiwan
\(^3\) Graduate Institute of Chinese Medicine, China Medical University, Taichung 40447, Taiwan
\(^4\) Tzu Chi Stem Cells Centre, Buddhist Tzu Chi General Hospital, Hualien 97002, Taiwan
\(^5\) Department of Stem Cell Applied Technology, Gwo Xi Stem Cell Applied Technology, Hsinchu 30261, Taiwan
\(^6\) Graduate Institute of Immunology, China Medical University, Taichung 40447, Taiwan
\(^7\) Department of Neurosurgery, China Medical University Beigan Hospital, Yunlin 65152, Taiwan
\(^8\) Department of Pathology, China Medical University Hospital, 2 Yuh-Der Road, Taichung 40447, Taiwan
\(^9\) Department of Medicine, China Medical University, Taichung 40447, Taiwan

Correspondence should be addressed to Shinn-Zong Lin, shinnzong@yahoo.com.tw and Horng-Jyh Harn, duke_harn@yahoo.com.tw

Received 25 May 2011; Revised 1 February 2012; Accepted 1 February 2012

Copyright © 2012 Po-Cheng Lin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Few rejuvenation and antiaging markers are used to evaluate food supplements. We measured three markers in peripheral blood to evaluate the antiaging effects of a food supplement containing placental extract. Samples were evaluated for CD34\(^+\) cells, insulin-like growth factor 1 (IGF1), and telomerase activity, which are all markers related to aging. To control the quality of this food supplement, five active components were monitored. In total, we examined 44 individuals who took the food supplement from 1.2 months to 23 months; the average number of CD34\(^+\) cells was almost 6-fold higher in the experimental group compared with the control group. Food supplement intake did not change serum IGF1 levels significantly. Finally, the average telomerase activity was 30% higher in the subjects taking this food supplement. In summary, our results suggest that the placental extract in the food supplement might contribute to rejuvenation and antiaging.

1. Introduction

Human placenta, also called ziheche in Chinese, has been used as a medicine in Korea and China for over 1,400 years due to its anti-aging and cosmetic properties [1]. In traditional oriental medicine, human placental extract has been used for wound healing. More recently, human placental extract was approved for treatment of abnormal liver function and menopausal symptoms and has no known toxic effects [2–4]. In both rodent and human cells in vitro, placental extract induces significant nitric oxide production, which is an important cellular mediator of tissue repair [5]. Placental extract contains several enzyme inhibitors, anticoagulant proteins, and antioxidants [6–8]. Furthermore, placental extract is a rich source of bioactive molecules such as hormones, proteins, lipids, nucleic acids, glycosaminoglycan, amino acids, vitamins, and minerals [2, 9]. It also contains many uncharacterized compounds believed to have various bioactivities involved in inhibition or delay of aging, inflammation, sunburn, mutagenicity, anaphylaxis, and oxidation [1].

Similar to human placenta, pig placenta has been used as a source of biomedical material. In fact, freeze-dried pig placenta contains equal or, in certain circumstances, higher nutritive properties than human placenta [10].

Hematopoietic stem cells (HSCs) represent a long-lived cell population that provides blood cells through hemopoiesis throughout the human lifespan. These cells are
identified by the surface marker CD34+. Hematopoiesis is regulated by a balance between self-renewal, proliferation, and differentiation. The capacity of HSCs to maintain their population through self-renewal replications throughout the aging process may imply that the HSC population itself does not age. However, diminishing proliferative capacity after serial transplantation [11], repeated exposure to irradiation [12], or age-related loss of telomeric DNA [13, 14] indicates that the life of HSCs may be limited. In humans, CD34+ stem cells represent about 0.01% to 0.03% of all cells in peripheral blood [15]. Regulation of hematopoiesis is altered during aging, impairing the ability of older people to respond appropriately to the physiological demand for blood cell replacement triggered by stimuli such as blood loss or cytoreductive chemotherapy [16]. In the peripheral blood and bone marrow, both the quantity and the clonogenic capacity of HSCs decline with age [15, 17, 18]. Furthermore, there are age-dependent differences in lineage maturation and cell-cycle phases [19], as well as an accumulation of genomic mutations and an imbalance in cytokine production [16]. Thus, HSCs are a key determinant in aging.

It is well established that a reduced concentration of insulin-like growth factor 1 (IGF1) plays a role in extending lifespan in invertebrates [20]. In mammalian evolution, IGF1 pathways have diverged from a single receptor to multiple receptors and even more complicated pathways and regulatory networks [21]. Insulin, IGF1, and growth hormone regulate metabolic pathways to control growth and differentiation [22]. This process depends mainly on the concentration of circulating IGF1. In mice, knockout of the insulin receptor (IR) pathway reduces lifespan and increases age-related diseases [23]. In contrast, both spontaneous and targeted genetic disruptions of the GH/IGF pathway are associated with small size (dwarfism), numerous indices of delayed aging, enhanced stress resistance, and a major increase in lifespan in mice [24]. These results suggest a role for the GH/IGF pathway in murine longevity. Furthermore, caloric restriction, well known to extend lifespan and slow down age-related pathology, is also associated with a reduced concentration of circulating IGF1 in nematodes, fruit flies, and mice [25]. On the other hand, reducing the concentration of IGF1 in humans, although protective against cancer, decreases the risk of cardiovascular disease and diabetes [26]. Moreover, human aging is associated with a decline in GH and IGF1 levels. It has been proposed that GH therapy may reverse some of the physiological features of aging [27].

Telomerase is pertinent to a cell’s potential for self-renewal and is required for long-term cellular proliferation and survival [28, 29]. Because human cells progressively lose telomerase activity, telomeric DNA is lost during successive rounds of DNA replication. Shortening of telomeres has been reported to correlate with cellular senescence [30, 31]. This indicates that replicating cells that lose the telomerase needed to maintain telomeres will eventually senesce [32].

The aim of the present study was to evaluate the rejuvenation and anti-aging effects of placental extract on healthy individuals by determining CD34+ cell counts, serum IGF1 levels, and monocyte telomerase activity in peripheral blood.

2. Materials and Methods

2.1. Source of 20070721-GX. The ingredients of 20070721-GX are pig placental extract, royal jelly, avocado oil, and wheat germ oil. 20070721-GX is a proprietary formulation available from Gwo Xi Stem Cell Applied Technology, Inc. To control the quality of each batch of the food supplement of 20070721-GX, the placental extract component is measured by ELISA. The evaluation includes concentrations of progesterone (Pgt), estriol (E3), estradiol (E4), human chorionic gonadotropin (HCG), human placental lactogen (hP/L), and total protein (tPrt). For each batch, the concentrations are 10 ± 5 mg/mL tPrt, 30 ± 5 ng/mL Pgt, 10 ± 5 ng/mL E3, 10 ± 5 ng/mL E4, 25 ± 5 mIU/mL HCG, and 0.15 ± 0.1 mg/L hP/L. Batches that fit these six indicators are chosen into chemistry, manufacturing, and controls (CMCs) manufacturing for standard operation and SGS examination.

2.2. Volunteers. This is a double-blind placebocontrolled trial. This research project was approved by the Institutional Review Board of China Medical University (DMR99-IRB-32). Forty-four subjects were recruited from China Medical University Hospital; 22 people have the product treatment (20070721-GX group), and the other 22 people were placebo treatment (Control group). The control group was 54.5% male, and the 20070721-GX group was 50% male. The mean age was 43 ± 16 years in the control group and 46 ± 9 years in the 20070721-GX group. Basic characteristics of individual subjects are listed in Tables 1 and 2.

2.3. Sample Collection. Individuals in the experimental group took three capsules (20070721-GX or placebo) daily for months. Peripheral blood samples from control and 20070721-GX subjects were drawn into BD Vacutainer CPT Tubes (Becton, Dickinson and Co., Franklin Lakes, NJ). Within 2 h of blood collection, blood samples were centrifuged at room temperature (18–25°C) in a horizontal rotor for 20 min at 1500 × g. After centrifugation, approximately half of the plasma was aspirated without disturbing the whitish cell layer consisting of mononuclear cells and platelets. The cell layer was collected with a Pasteur pipette and transferred to a microcentrifuge tube.

2.4. Flow Cytometry of CD34+ Hematopoietic Stem and Progenitor Cells. Hematological Cell Counts. The total number of CD34+ cells from peripheral blood samples was measured by flow cytometry (Trucount, BD). For the two-platform method, results were expressed as % CD34+ cells, and the absolute number of CD34+ cells per microliter was calculated as % CD34+ cells × white blood cells × 103 μL. For the one-platform method, the number of CD34+ cells per microliter was calculated as CD34+ cells/μL = (number of CD34+ cells × bead count per test × dilution factor)/number of beads collected.

2.5. Sample Acquisition. Cells were acquired on a three-color FACSCalibur flow cytometer (BD) equipped with a 488-nm argon laser and analyzed with CellQuest 3.1 software. The
Table 1: Demographics, CD34+ Cells, telomerase activity, and IGF1 concentration in control subjects.

| Sample No. | Age (y) | Gender | Duration (m) | CD34 (%) | Telomerase (U) | IGF-1 (ng/mL) |
|------------|---------|--------|--------------|----------|----------------|---------------|
| Control    |         |        |              |          |                |               |
| 1          | 19      | m      | 0            | 0.10     | 0.14           | 138.04        |
| 2          | 20      | f      | 0            | 0.10     | 0.08           | 150.61        |
| 3          | 20      | f      | 0            | 0.20     | 0.08           | 128.54        |
| 4          | 21      | m      | 0            | 0.20     | 0.10           | 78.95         |
| 5          | 21      | f      | 0            | 0.20     | 0.08           | 145.85        |
| 6          | 22      | m      | 0            | 0.30     | 0.08           | 71.82         |
| 7          | 32      | f      | 0            | 0.70     | 0.15           | 133.39        |
| 8          | 39      | f      | 0            | 0.50     | 0.09           | 129.83        |
| 9          | 41      | m      | 0            | 0.10     | 0.13           | 165.89        |
| 10         | 46      | m      | 0            | 0.20     | 0.13           | 77.93         |
| 11         | 47      | f      | 0            | 0.30     | 0.15           | 114.37        |
| 12         | 48      | m      | 0            | 0.10     | 0.10           | 60.95         |
| 13         | 53      | m      | 0            | 0.20     | 0.08           | 141.97        |
| 14         | 53      | m      | 0            | 0.30     | 0.08           | 72.16         |
| 15         | 54      | f      | 0            | 0.20     | 0.11           | 95.00         |
| 16         | 56      | f      | 0            | 0.30     | 0.10           | 104.71        |
| 17         | 56      | m      | 0            | 0.05     | 0.11           | 141.96        |
| 18         | 57      | f      | 0            | 0.10     | 0.09           | 153.90        |
| 19         | 58      | m      | 0            | 0.50     | 0.12           | 109.15        |
| 20         | 60      | m      | 0            | 0.50     | 0.08           | 109.91        |
| 21         | 64      | m      | 0            | 0.15     | 0.09           | 110.00        |
| 22         | 64      | f      | 0            | 0.05     | 0.08           | 108.00        |
| **Average** | 43      |        |              | 0.24     | 0.10           | 115.59        |
| **SD**     | 15.8    |        |              | 0.17     | 0.02           | 29.37         |

*The ratio of male.

2.7. Analysis of Telomerase Activity (TRAP Assay). Analysis of telomerase activity was assessed using the telomeric repeat amplification protocol (TRAP) assay [34] according to the manufacturer’s protocol (Roche, Pleasanton, CA, USA). The TRAP assay was performed on 30 μg protein per sample extract. Thirty cycles of PCR were performed at 94°C for 30 s, 50°C for 30 s, and 72°C for 90 s. Sample absorbance was measured at 450 nm using a PowerWave × Microplate ELISA Reader (Bio-Tek Instruments).

2.8. Statistical Analysis. Univariable, multivariable, and stepwise regression analyses with two-sided t tests were used to evaluate the effect of 20070721-GX, gender, and age on changes in number of CD34+ progenitor cells, IGF1 concentration, and telomerase activity; P < 0.05 was considered statistically significant.

3. Results

3.1. 20070721-GX-Induced Increase in Progenitor CD34+ Cells. Circulating cells were analyzed according to cell-surface markers, with progenitor cells defined as CD34+. The number of CD34+ progenitor cells differed significantly between the control and 20070721-GX groups (Figure 1(a), Table 3, P < 0.001). The average level of CD34+ progenitor cells was about 6-fold higher in the 20070721-GX group compared with the control group (Tables 1 and 2). More than
Table 2: Demographics, CD34+ Cells, telomerase activity, and IGF1 concentration in 20070721-GX subjects.

| Sample No. | Age (y) | Gender | Duration (m) | CD34 (%) | Telomerase (U) | IGF-1 (ng/mL) |
|------------|---------|--------|--------------|----------|----------------|---------------|
| 20070721-GX |         |        |              |          |                |               |
| 23         | 27      | m      | 2            | 4.60     | 0.27           | 68.08         |
| 24         | 27      | f      | 2            | 3.90     | 0.12           | 172.53        |
| 25         | 35      | f      | 2            | 1.35     | 0.14           | 113.52        |
| 26         | 39      | f      | 8            | 1.70     | 0.14           | 74.43         |
| 27         | 40      | m      | 12           | 1.70     | 0.13           | 117.07        |
| 28         | 41      | m      | 3            | 1.00     | 0.10           | 133.14        |
| 29         | 43      | m      | 12           | 0.60     | 0.08           | 79.82         |
| 30         | 44      | f      | 5            | 0.70     | 0.09           | 99.85         |
| 31         | 44      | f      | 6            | 0.30     | 0.09           | 142.48        |
| 32         | 45      | f      | 1.7          | 5.95     | 0.10           | 113.79        |
| 33         | 46      | f      | 2            | 0.20     | 0.12           | 97.65         |
| 34         | 46      | f      | 8            | 0.40     | 0.23           | 129.58        |
| 35         | 46      | m      | 23           | 1.70     | 0.22           | 118.71        |
| 36         | 48      | m      | 1.2          | 0.30     | 0.13           | 75.49         |
| 37         | 49      | m      | 14           | 0.35     | 0.15           | 88.34         |
| 38         | 51      | m      | 12           | 1.25     | 0.13           | 79.32         |
| 39         | 54      | f      | 12           | 0.30     | 0.12           | 99.85         |
| 40         | 57      | m      | 12           | 1.10     | 0.10           | 126.29        |
| 41         | 58      | f      | 8            | 1.10     | 0.08           | 155.78        |
| 42         | 58      | f      | 11           | 0.60     | 0.11           | 74.34         |
| 43         | 59      | m      | 2            | 0.65     | 0.10           | 93.82         |
| 44         | 61      | m      | 3            | 1.50     | 0.15           | 123.91        |

Average 46 50%\(^{\text{a}}\) 1.42 0.13 108.08
SD 9.2 1.47 0.05 27.75

\(^{\text{a}}\)The ratio of male.

half of 20070721-GX subjects (12/22, 54.5%) had more than 1% CD34+ progenitor cells in peripheral blood. Regression analysis showed that 20070721-GX significantly increased the number of CD34+ progenitor cells in peripheral blood. However, age, gender, and the duration of 20070721-GX intake did not affect the amount of CD34+ progenitor cells (Table 3).

3.2. 20070721-GX-Induced Increase in Telomerase Activity. With sufficient telomerase enzyme, it is possible to prevent cells from dying. The clinical trials showed that telomerase activity differed significantly between the control and 20070721-GX groups (Figure 1(b), Table 4, \(P = 0.016\)). The average telomerase activity increased about 30% in the 20070721-GX group compared with the control group (Tables 1 and 2). Regression analysis showed that 20070721-GX significantly increased telomerase activity in subjects. The duration of 20070721-GX intake had a small but significant effect on the increase in telomerase activity (\(\beta = 0.003, P = 0.022\)) using univariable regression analysis; however, this was not significant after adjusting other variables, and duration was consequently excluded from stepwise regression analysis. Age and gender did not affect telomerase activity (Table 4).

3.3. Effect of 20070721-GX on Serum IGF1 Concentration. To evaluate IGF1 concentrations in this cohort, we used ELISA. Serum IGF1 ranged from 60 to 165 ng/mL (mean, 115 ± 30 ng/mL) among control subjects and from 68 to 172 ng/mL (mean, 108 ± 28 ng/mL) among the 20070721-GX subjects (Tables 1 and 2). Although the average IGF1 concentration in 20070721-GX subjects decreased slightly, it did not reach statistical significance (Figure 1(c), \(P = 0.40\)).

4. Discussion

To control the quality of each batch of the food supplement of 20070721-GX, the placental extract component is measured by ELISA. The evaluation includes concentrations of progesterone (Pgt), estriol (E3), estradiol (E4), human chorionic gonadotrophic (HCG), human placental lactogen (hPL), and total protein (tPrt). For each batch, the concentrations are 10 ± 5 mg/mL tPrt, 30 ± 5 ng/mL Pgt, 10 ± 5 mg/mL E3, 10 ± 5 mg/mL E4, 25 ± 5 mIU/mL HCG, and 0.15 ± 0.1 mg/L hPL. Batches that fit these six indicators are chosen into chemistry, manufacturing, and controls (CMCs) manufacturing for standard operation and SGS examination. Because this placental extract is derived from pig at 8 weeks pregnancy (full term is 20 weeks), it is full of nutrition and
The peptide concentrations are relatively high compared with other placental extracts collected at full term.

In our study, 95% (21/22) of individuals had an increase of more than 20% of CD34+ stem cells in peripheral blood compared with the control group (Tables 1 and 2). The enhancing effect was being observed in two elderly people (Tables 1 and 2). One subject was 59 years old (no. 43) and showed a 2.7-fold increase over control, and another was 61 years old (no. 44) with a 6.25-fold increase. In human, CD34+ cells can increase by 15% in young people but not in elderly people after taking exercise [15]. However, the food supplement 20070721-GX can increase health not only in the young but also in middle-aged or older people. In the present study, the CD34+ stem cells in peripheral blood of subjects that took 20070721-GX (1.42 ± 1.50%) were significantly higher than in control subjects (0.24 ± 0.17%, P = 0.001) (Tables 1 and 2).

In addition, the CD34+ status of two young individuals increased 7 times and 24 times after taking the supplement for only 2 months in comparison to the mean of the control group (Tables 1 and 2). This result indicates that duration of food supplement intake was not closely related to the increase in CD34+ cells (Table 3).

Other issues arise from this result. Is the increase in CD34+ cells enough to repair injured cells or cause rejuvenation? According to a study by Khosrotehrani et al. [35], a total of 701 male (XY+) microchimeric cells were identified (mean [SD], 227 (128) XY+ cells per million maternal cells [0.02%]). Interestingly, in maternal epithelial tissues (thyroid, cervix, intestine, and gallbladder), 14% to 60% of XY+ cells expressed cytokeratin. This result implied that only a small amount of stem cells from the son is needed to repair injured cells derived from maternal tissues. Stem cells can not only transdifferentiate different organs, but they are also involved in renewal and proliferation. Because this food supplement increased CD34+ stem cells by 20% in 95% of subjects, we suggest that it could play a role in repairing injured tissue. Direct evidence, however, requires further study.

Using the TRAP assay, we found that 2.2 to 2.3-fold (to 0.22–0.23 U) increases in telomerase activity after two 46-year-old subjects (nos. 34 and 35) took 20070721-GX for 8 or

---

**Table 3: Regression analysis for CD34+ cells.**

| Variable       | Univariable |         | Multivariable |         | Stepwise |
|----------------|-------------|---------|---------------|---------|----------|
|                | β (95% CI)  | P       | β (95% CI)    | P       | β (95% CI) | P       |
| 20070721-GX    | 1.177 (0.527 ~ 1.827) | 0.001*  | 1.732 (0.851 ~ 2.613) | <0.001*  | 1.177 (0.527 ~ 1.827) | 0.001*  |
| Duration (months) | 0.036 (~0.033 ~ 0.105) | 0.296  | −0.068 (~0.151 ~ 0.015) | 0.106  | 0.052 (~0.597 ~ 0.700) | 0.873  | Excluded |
| Male           | −0.153 (~0.899 ~ 0.592) | 0.680  | 0.052 (~0.597 ~ 0.700) | 0.873  | 0.052 (~0.597 ~ 0.700) | 0.873  | Excluded |
| Age (years)    | −0.015 (~0.043 ~ 0.014) | 0.306  | −0.018 (~0.043 ~ 0.007) | 0.157  | 0.052 (~0.597 ~ 0.700) | 0.873  | Excluded |

*P < 0.05 considered statistically significant.
23 months (Table 2). Although an increase in telomerase activity has an antisenescence effect, increasing telomerase activity may also cause tumorigenesis [36, 37]. Therefore, we measured the telomerase activity in two malignant brain tumor cell lines: DBTRG and 8401. Telomerase activity in these cells was 1.8 U and 2.7 U, respectively, which is much higher than the average activity of 0.13 U in the experimental subjects. Furthermore, according to Fu and Chen [38], telomerase activity in normal bone marrow is 0.3 U, which is close to the increased level of activity in our experimental subjects. These results suggest that this food supplement does not cause tumorigenesis.

Telomerase activity is inversely related to senescence. IGF1 is associated with rejuvenation, and CD34+ cells are related to injured organ repair and anti-inflammatory activity. Thus, it is important to monitor the phenotypic features of people taking food supplements. Skin became more fine and tight, and wrinkles decreased in some people. In addition, more energy, increased sexual impulses, and improvement of menstrual disorder symptoms were described by others. These features are related to rejuvenation and antisenescence. Furthermore, decreased serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase liver enzymes and improved nasal allergies, arthritis, and lower back pain were also observed. These features could be related to anti-inflammatory activity and injured cell repair. These phenotypic features are subjective and only represent a preliminary finding but are worth noting when designing future studies.

In our study, taking the food supplement increased the number of CD34+ cells and increased telomerase activity, but decreased IGF1 in the same subjects (nos. 23, 25, 26, 33, 36, 37, 38, 39, and 42; 9/22 = 40%). Thus, an important issue is whether the placental extract affects the same cellular target in each case. Because there are so many compounds in placental extract, it is possible that different targets of different compounds may lead to three different effects. However, because CD34+ stem cells have a relatively high telomerase activity [39], these effects are likely to be linked in placental extract that likely affects both. However, because IGF1 is an independent biomarker for antisenescence, none of the subjects that took the food supplement had only a decrease in IGF1. The placental extract should be fractionated to determine which components are responsible for which effects.

### Table 4: Regression analysis for telomerase activity.

| Variable          | Univariable | Multivariable | Stepwise |
|-------------------|-------------|---------------|----------|
|                   | β (95% CI)  | P             | β (95% CI) | P     | β (95% CI) | P    |
| 20070721-GX       | 0.030 (0.006 ~ 0.053) | 0.016* | 0.022 (0.011 ~ 0.055) | 0.186 | 0.030 (0.006 ~ 0.053) | 0.016* |
| Duration (months) | 0.003 (0.000 ~ 0.005) | 0.022* | 0.001 (0.002 ~ 0.004) | 0.372 | Excluded   |       |
| Male              | 0.010 (0.015 ~ 0.035) | 0.436 | 0.011 (0.013 ~ 0.035) | 0.349 | Excluded   |       |
| Age (years)       | 0.000 (0.001 ~ 0.001) | 0.422 | −0.001 (−0.002 ~ 0.000) | 0.172 | Excluded   |       |

*P < 0.05 considered statistically significant.

### Abbreviations

- *IGF1*: Insulin-like growth factor 1
- *HSC*: Hematopoietic stem cells
- *IR*: Insulin receptor
- *Pgt*: Progesterone
- *E3*: Estriol
- *E4*: Estriol
- *HCG*: Human choronic gonodotropic
- *hPL*: Human placental lactogen
- *tPrt*: Total protein
- *CMC*: Chemistry manufacturing, and controls
- *ISHAGE*: International society of hematotherapy and graft engineering
- *TMB*: 3,3′,5,5′-Tetramethylbenzidine
- *TRAP*: Telomeric repeat amplification protocol

### Authors’ Contribution

The first two authors contributed equally to this work.

### Acknowledgment

This work was supported by grants from the Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH100-TD-B-111-004) and China Medical University (no. 097426K9).

### References

1. H. J. Kim, J. W. Lee, Y. L. Kim, and M. H. Lee, “The effect of placental extract on the expression of tyrosinase, TRP-1 and TRP-2 in SK30 melanoma cells,” *Korean Journal of Dermatology*, vol. 41, no. 12, pp. 1612–1618, 2003.
2. S. Togashi, N. Takehashi, M. Iwama, S. Watanabe, K. Tamagawa, and T. Fukui, “Antioxidative collagen-derived peptides in human-placenta extract,” *Placenta*, vol. 23, no. 6, pp. 497–502, 2002.
3. G. Tonello, M. Daglio, N. Zaccarelli, E. Sottofattori, M. Mazzei, and A. Balbi, “Characterization and quantitation of the active polynucleotide fraction (PDRN) from human placenta, a tissue repair stimulating agent,” *Journal of Pharmaceutical and Biomedical Analysis*, vol. 14, no. 11, pp. 1555–1560, 1996.
4. Y. K. Lee, H. H. Chung, and S. B. Kang, “Efficacy and safety of human placenta extract in alleviating climacteric symptoms: prospective, randomized, double-blind, placebo-controlled
trial,” *Journal of Obstetrics and Gynaecology Research*, vol. 35, no. 6, pp. 1096–1101, 2009.

[5] A. Sabapatha, C. Gercel-taylor, and D. D. Taylor, “Specific isolation of placenta-derived exosomes from the circulation of pregnant women and their immunoregulatory consequences,” *American Journal of Reproductive Immunology*, vol. 56, no. 5–6, pp. 345–355, 2006.

[6] K. C. Hooper, “The action of inhibitors on enzymes from human placenta,” *The Journal of Physiology*, vol. 148, pp. 283–290, 1959.

[7] M. Uszynski, “Anticoagulant activity of peptides from the human placenta,” *Thrombosis Research*, vol. 16, no. 5–6, pp. 689–694, 1979.

[8] H. Mochizuki and T. Kada, “Antimutagenic action of mammalian placental extracts on mutations induced in Escherichia coli by UV radiation, gamma-rays and N-methyl-N-nitro-N-nitrosoguanidine,” *Mutation Research*, vol. 95, no. 2–3, pp. 457–474, 1982.

[9] S. Watanabe, S. I. Togashi, N. Takahashi, and T. Fukui, “L-tryptophan as an antioxidant in human placenta extract,” *Journal of Nutritional Science and Vitamins*, vol. 48, no. 1, pp. 36–39, 2002.

[10] A. Jang, C. Jo, I.-J. Kim, and M. Lee, “Nutritional quality of dried pig placenta,” *Journal of Food Science and Nutrition*, vol. 12, pp. 89–94, 2007.

[11] D. E. Harrison, C. M. Astle, and J. A. Delaittre, “Loss of proliferative capacity in immunohemopoietic stem cells caused by serial transplantation rather than aging,” *Journal of Experimental Medicine*, vol. 147, no. 5, pp. 1526–1531, 1978.

[12] D. R. Boggs, J. C. Marsh, P. A. Chervenick, G. E. Cartwright, and M. M. Wintrobe, “Factors influencing hematopoietic spleen colony formation in irradiated mice. 3. The effect of repetitive irradiation upon proliferative ability of colony-forming cells,” *Journal of Experimental Medicine*, vol. 126, no. 5, pp. 871–880, 1967.

[13] H. Vaziri, W. Dragowska, R. C. Allsopp, T. E. Thomas, C. B. Harley, and P. M. Lansdorp, “Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 21, pp. 9857–9860, 1994.

[14] G. M. Baerlocher, A. Roth, and P. M. Lansdorp, “Evidence for a mitotic clock in human hematopoietic stem cells,” *Annals of the New York Academy of Sciences*, vol. 960, pp. 44–48, 2003.

[15] R. G. Shaffer, S. Greene, A. Arshi et al., “Effect of acute exercise on endothelial progenitor cells in patients with peripheral arterial disease,” *Vascular Medicine*, vol. 11, no. 4, pp. 219–226, 2006.

[16] G. Rothstein, “Disordered hematopoiesis and myelodysplasia in the elderly,” *Journal of the American Geriatrics Society*, vol. 51, no. 3, supplement, pp. S22–S26, 2003.

[17] Y. Egusa, Y. Fujitani, M. Yamakido, and M. Uszynski, “Age-effect of human peripheral blood stem cells,” *Oncology Reports*, vol. 5, no. 2, pp. 397–400, 1998.

[18] R. Morelli, S. Tesei, L. Costarelli et al., “Age- and gender-related alterations of the number and clonogenic capacity of circulating CD34+ progenitor cells,” *Biogerontology*, vol. 6, no. 3, pp. 185–192, 2005.

[19] C. C. Lee, M. D. Fletcher, and A. F. Tarantal, “Effect of age on the frequency, cell cycle, and lineage maturation of rhesus monkey (Macaca mulatta) CD34+ and hematopoietic progenitor cells,” *Pediatric Research*, vol. 58, no. 2, pp. 315–322, 2005.

[20] C. Kenyon, “The plasticity of aging: insights from long-lived mutants,” *Cell*, vol. 120, no. 4, pp. 449–460, 2005.

[21] C. Kenyon, “A conserved regulatory system for aging,” *Cell*, vol. 105, no. 2, pp. 165–168, 2001.

[22] J. Dupont and D. LeRoith, “Insulin and insulin-like growth factor I receptors: similarities and differences in signal transduction,” *Hormone Research*, vol. 55, supplement 2, pp. 22–26, 2001.

[23] D. LeRoith and O. Gavrilova, “Mouse models created to study the pathophysiology of Type 2 diabetes,” *International Journal of Biochemistry and Cell Biology*, vol. 38, no. 5–6, pp. 904–912, 2006.

[24] M. S. Bonkowski, J. S. Rocha, M. M. Masternak, K. A. Al Regaiey, and A. Bartke, “Targeted disruption of growth hormone receptor interferes with the beneficial actions of calorie restriction,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 20, pp. 7901–7905, 2006.

[25] A. Bartke, “Minireview: role of the growth hormone/insulin-like growth factor system in mammalian aging,” *Endocrinology*, vol. 146, no. 9, pp. 3718–3723, 2005.

[26] J. Yang, M. Anzo, and P. Cohen, “Control of aging longevity by IGF-I signaling,” *Experimental Gerontology*, vol. 40, no. 11, pp. 867–872, 2005.

[27] M. L. Vance, “Can growth hormone prevent aging?” *The New England Journal of Medicine*, vol. 348, no. 9, pp. 779–780, 2003.

[28] S. J. Morrison, K. R. Prowse, P. Ho, and I. L. Weissman, “Telomerase activity in hematopoietic cells is associated with self-renewal potential,” *Immunity*, vol. 5, no. 3, pp. 207–216, 1996.

[29] H. W. Lee, M. A. Blasco, G. J. Gottlieb, J. W. Horner II, C. W. Greider, and R. A. DePinho, “Essential role of mouse telomerase in highly proliferative organs,” *Nature*, vol. 392, no. 6676, pp. 569–574, 1998.

[30] R. C. Allsopp, H. Vaziri, C. Patterson et al., “Telomere length predicts replicative capacity of human fibroblasts,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 21, pp. 10114–10118, 1992.

[31] T. von Zglinicki, G. Saretzki, W. Docke, and C. Lotze, “Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence?” *Experimental Cell Research*, vol. 220, no. 1, pp. 186–193, 1995.

[32] G. A. Ulaner, J. F. Hu, T. H. Yu, L. C. Giudice, and A. R. Hoffman, “Telomerase activity in human development is regulated by human telomerase reverse transcriptase (hTERT) transcription and by alternate splicing of hTERT transcripts,” *Cancer Research*, vol. 58, no. 18, pp. 4168–4172, 1998.

[33] J. W. Gratama, A. Orfao, D. Barnett et al., “Flow cytometric enumeration of CD34+ hematopoietic stem and progenitor cells. European working group on clinical cell analysis,” *Cytometry*, vol. 34, no. 3, pp. 128–142, 1998.

[34] N. W. Kim, M. A. Piatsyzek, K. R. Prowse et al., “Specific association of human telomerase activity with immortal cells and cancer,” *Science*, vol. 266, no. 5193, pp. 2011–2015, 1994.

[35] K. Khosrotehrani, K. L. Johnson, D. H. Cha, R. N. Salomon, and D. W. Bianchi, “Transfer of fetal cells with multilineage potential to maternal tissue,” *Journal of the American Medical Association*, vol. 292, no. 1, pp. 75–80, 2004.

[36] T. X. Chen, Y. Y. Xiong, and L. Liu, “Expression and significance of telomerase reverse transcriptase and its regulators in non-small cell lung carcinoma,” *Ai Zheng*, vol. 23, no. 3, pp. 273–277, 2004.

[37] S. M. Yang, D. C. Fang, J. L. Yang, L. Chen, Y. H. Luo, and G. P. Liang, “Antisense human telomerase reverse transcriptase
could partially reverse malignant phenotypes of gastric carcinoma cell line in vitro, "European Journal of Cancer Prevention, vol. 17, no. 3, pp. 209–217, 2008.

[38] C. C. Fu and Z. X. Chen, "Study of telomerase activity in bone marrow cells from patients with myelodysplastic syndromes," Zhongguo Shi Yan Xue Ye Xue Za Zhi, vol. 9, no. 4, pp. 303–306, 2001.

[39] H. Handa, T. Matsushima, N. Nishimoto et al., "Flow cytometric detection of human telomerase reverse transcriptase (hTERT) expression in a subpopulation of bone marrow cells," Leukemia Research, vol. 34, no. 2, pp. 177–183, 2010.