Telomerase Activity and Telomerase Reverse Transcriptase Expression Induced by Selenium in Rat Hepatocytes[1]

RI-AN YU[*2], HUA-JIE CHEN[Δ], LING-FEI HE[#], BING CHEN[*], AND XUE-MIN CHEN[Δ]

[*]Department of Occupational and Environmental Health, School of Public Health, Guangdong Pharmaceutical University, Guangzhou 510310, Guangdong, China; [Δ]Department of Occupational and Environmental Health, MOE Key Laboratory of Environment and Health, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, China; [#]Department of Dental Medicine, The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou 510080, Guangdong, China

Objective To investigate the effects of sodium selenite on telomerase activity, apoptosis and expression of TERT, c-myc and p53 in rat hepatocytes. **Methods** Selenium at doses of 2.5, 5.0, and 10 μmol/kg was given to SD rats by gavage. In rat hepatocytes, telomerase activity was measured by the telomeric repeat amplification protocol (TRAP), apoptosis was detected by flow cytometry, and expressions of telomerase reverse transcriptase (TERT), c-myc and p53 were analyzed by reverse transcription-polymerase chain reaction (RT-PCR). c-Myc and P53 proteins were detected by immunochemistry. **Results** Selenium at doses of 2.5, 5.0, and 10 μmol/kg significantly increased hepatocellular telomerase activity and induced apoptosis in a dose-dependent manner. Although selenium at doses of 2.5, 5.0, and 10 μmol/kg displayed no obvious enhancing effect on the TERT mRNA expression in rat hepatocytes ($P>0.05$), it significantly increased the c-myc mRNA and p53 mRNA expression at the dose of 10 μmol/kg ($P<0.05$). Selenium at doses of 5.0 and 10 μmol/kg obviously increased the content of P53 protein in rat hepatocytes, but only at the dose of 10 μmol/kg, it significantly promoted the value of c-Myc protein in them. **Conclusion** Selenium can slightly increase telomerase activity and TERT expression, and significantly induce apoptosis and over-expression of c-myc and p53 at relatively high doses. The beneficial effects of selenium on senescence and aging may be mediated by telomerase activation and expression of TERT, c-myc, and p53 in rat hepatocytes.

Key words: Selenium; Telomerase; Telomerase reverse transcriptase; c-myc; p53

INTRODUCTION

Telomerase biology has become one of the most active fields in aging research. Telomere, the end of chromosome structures with tandem repeats, undergoes shortening in human somatic proliferating cells and is considered a biological clock in the countdown of cellular replication senescence. Telomerase, a reverse transcriptase that can replenish replication-dependent loss of telomere DNA, is expressed at a low or non-detectable level in most human somatic cells. Inactivation of telomerase contributes to telomere loss during cellular senescence and aging. Telomerase regulation, which relates to telomere length maintenance, has constituted a critical factor in the control of aging and age-related disorders[1].

Selenium (Se), a universal essential trace element for mammals, is important for many cellular processes. Aging is associated with a progressive decrease in selenium status[2], and selenium supplementation at doses of 0.5 and 2.5 μmol/L significantly extends the telomere length of hepatocytes L-02, thus prolonging their life-span[3]. The mechanism of selenium in resisting senescence

[1]This work is supported by the Scientific Foundation and Teacher's Foundation of Guangdong Pharmaceutical University (No.2006GGW01), and the National Natural Science Foundation of China (No.30271110).

[2]Correspondence should be addressed to Dr. Ri-An YU, Department of Occupational and Environmental Health, School of Public Health, Guangdong Pharmaceutical University, Guangzhou 510310, Guangdong, China. Tel:86-20-34055940. Fax: 86-20-34055355. E-mail: yurian_tj@yahoo.com.cn

Biographical note of the first author: Ri-An YU, male, born in 1964, Ph. D., professor, majoring in environmental and molecular toxicology.

0895-3988/2009
CN 11-2816/Q

in hepatocytes may be related to its antioxidative property, because cellular replicable senescence is heterogeneous and the rates of aging and telomere shortening depend on the balance between oxidative stress and antioxidative defense[3-4]. Liu *et al*.[5] hold that the selenite-elongated telomere is related to the activation of telomerase, because sodium selenite significantly increases cellular telomerase activity and hTERT gene expression level. The telomere length of L-02 cells is also extended after 4-week-cultivation with sodium selenite. The telomere is significantly longer and telomerase activity is higher in Se-treated Saccharomyces cerevisiae cells[6].

Although these *in vitro* studies displayed that selenium could induce telomerase activity and elongate telomere length, the detailed mechanism of selenium underlying anti-senescence property needs to be further studied. In this *in vivo* research, we studied the effects of selenium on telomerase activity, apoptosis, and expression of TERT, c-myc and p53 in rat hepatocytes.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (weighing approximately 200±30 g) used in this study were housed in polycarbonated cages with compressed fiber bedding with free access to commercial pelleted diet and water. The animals were divided into 4 groups (n=5). Rats in the first (control) group received no selenite treatment but 0.9% NaCl, while rats in the second, third, and forth groups received 2.5 μmol/kg, 5.0 μmol/kg and 10 μmol/kg Na_2SeO_3, respectively. Na_2SeO_3 was prepared in 0.9% NaCl and administered by *gavage*. The *gavage* volume was 1 mL/200g body weight. The animals were sacrificed in 48 h after the initial treatment with their livers removed immediately for use.

Telomerase Activity Assay

Telomerase activity was examined following the telomeric repeat amplification protocol (TRAP), using a TRAPeze® XL telomerase detection kit (Chemicon International). Briefly, hepatocytes were separated by trypsinization, resuspended in CHAPS lysis buffer and incubated for 30 min on ice. Hepatocytes (1.0×10^5) were used for each telomerase assay. After centrifugation at 12 000 g for 30 min at 4 ℃, supernatant was used as a cell extract. TRAP assay was performed with 1.0 μg cell extract. The assay specificity for active telomerase was determined by inclusion of heat-inactivated samples, in which the extract was heat-inactivated at 85 ℃ for 10 min before TRAP assay. The assay was repeated 2-4 times, and the activity was semi-quantified by fluorescence measurement, as recommended by the manufacturer. Their mean value was taken as the telomerase activity of samples.

Flow Cytometry

It has been well established that DNA fragmentation during apoptosis can lead to extensive loss of DNA content and result in a distinct sub-G1 peak when analyzed by flow cytometry. Selenium-induced apoptosis was analyzed and evaluated by determining sub-G1 cells. At the end of various designated treatments, cells were washed, fixed and permeated with 70% ice-cold ethanol at 4 ℃ for 2 h. Cells were then incubated with a freshly prepared propidium iodide (PI) staining buffer (0.1% Triton X-100, 200 μg/mL RNase A, and 20 μg/mL PI in PBS) for 15 min at 37 ℃, followed by flow cytometry analysis of 20 000 cells in each group. Histogram was abstracted and percentage of cells in the sub-G1 phase was then calculated and expressed as the percentage of apoptotic cells.

Isolation of RNA and Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis

Total RNA was isolated from cells using Trizol (GIBCO，Scotland), according to its manufacturer's instructions. First strand cDNA synthesis with oligo(dt) primers was performed using M-MLV (reverse transcriptase). The primer sequences and PCR product sizes are shown in Table 1. The PCR products were subjected to agarose gel electrophoresis and observed with ethidium bromide staining. The quality of cell mRNA was confirmed by the intensity of β-actin.

Immunochemistry

Tissue sections were deparaffinized in xylene, hydrated in grated ethanol continuously, and then covered with 3% hydrogen peroxide in PBS to block the endogenous peroxidase activity for 10 min. The sections were pretreated in a citrate buffer (0.01 mol/L, pH 6.0) under microwave heating for 20 min to retrieve antigen. Normal goat serum was added onto the sections for 30 min at room temperature, and the sections were incubated overnight at 4 ℃ with monoclonal primary antibody, anti-c-Myc, anti-P53 (Santa Cruez), respectively. PBS (0.01 mol/L, pH 7.4) was used as a substitute for monoclonal primary antibody of anti-c-Myc and anti-P53, respectively, in control sections (blank controls). SP kit was purchased from Beijing Zhongshan Biotechnology

TABLE 1

RT-PCR Primer Sequences

Target cDNA	Primer Sequences	Product Size (bp)
TERT	F 5′-GAC ATG GAG AAC AAG CTG TTT GC-3′ R 5′-ACA GGG AAG TTC ACC ACT GTC-3′	185
c-myc	F 5′-AAC TTA CAA TCT GCG AGC CA-3′ R 5′- AGC AGC TCG AAT TTC TTC CAG ATA T-3′	342
p53	F 5′- TTC CCT CAA TAA GCT GTT CTG CC-3′ R 5′- TGC TCT CTT TGC ACT CCC TGG-3′	538
β-actin	F 5′- GAG ACC TTC AAG ACC CCA GCC-3' R 5′- TCG GGG CAT CGG AAC CGC TCA-3'	404

Ltd (China). After the sections were washed three times in PBS (5 min each time), biotinylated goat secondary antibody was added for 30 min followed by avidin-biotinylated peroxidase complex for another 30 min at room temperature. After washing with PBS, the sections were stained with diaminobenzidine (DAB) and hydrogen peroxide for 3 min, and stain reaction was terminated with tap water.

Statistical Analysis

Data were expressed as $\bar{x} \pm s$. Statistical analysis was performed with ANOVA (SPSS Software). $P<0.05$ was considered statistically significant.

RESULTS

Effect of Selenium on Telomerase Activity and Apoptosis of Hepatocytes

Selenium at doses of 2.5, 5.0, and 10 μmol/kg increased hepatocellular telomerase activity in a dose-dependent manner (r=0.8451, $P<0.05$), but statistical analysis showed an insignificant difference in three selenium-treated groups; and no significant difference was observed between the selenium-treated and control groups. The effect of sodium selenite on telomerase activity and hepatocellular apoptosis detected by flow cytometry is shown in Table 2. There was a statistically significant difference in apoptotic rates between the selenium-treated groups at the doses of 5.0 and 10 μmol/kg and the control group and the dose-response relationship was obvious. Statistical analysis yielded a relative coefficient value of 0.9894 ($P<0.01$). The relative coefficient value for telomerase activity and apoptosis rate was 0.8876 ($P<0.05$).

TABLE 2

Effect of Selenium on Telomerase Activity and Apoptosis of Hepatocytes ($\bar{x} \pm s$, n=5)

Groups	Telomerase Activity	Apoptosis Rate (%)
Control (0 μmol/kg)	42.14±6.09	4.16±0.74
Na_2SeO_3 (2.5 μmol/kg)	56.57±8.61	5.77±0.78
Na_2SeO_3 (5.0 μmol/kg)	61.03±15.68	7.95±0.68*
Na_2SeO_3 (10 μmol/kg)	62.97±17.06	10.16±0.97**

Note. *$P<0.05$, **$P<0.01$ *vs* control group.

Effect of Selenium on TERT, c-myc and p53 Expression in Hepatocytes

RT-PCR showed that selenium at the doses of 2.5, 5.0, and 10 μmol/kg did not significantly increase the TERT mRNA expression ($P>0.05$), but at the dose of 10 μmol/kg it significantly increased the c-myc mRNA and p53 mRNA expression in rat hepatocytes of the selenium-treated and control groups ($P<0.05$, Table 3, Figs. 1-3).

The c-myc mRNA and p53 mRNA integrated optical density (IOD) induced by selenium at the doses of 2.5, 5.0 μmol/kg was higher in the selenium treated groups than in the control group, suggesting that a relatively high selenium dose can induce c-myc and p53 expression.

Effect of Selenium on c-myc and P53 Protein Expression in Rat Hepatocytes

c-Myc and P53 proteins were mainly expressed in cytoplasm of hepatocytes (Fig. 4). Selenium increased the P53 protein level at doses of 5.0 and 10 μmol/kg, but significantly increased the c-Myc protein expression level in rat hepatocytes only at the dose of 10 μmol/kg.

TABLE 3

Effect of Selenium on TERT, c-myc and p53 Expression in Hepatocytes ($\bar{x} \pm s$, n=5)

Groups	TERT/actin	c-myc/actin	p53/actin
Control (0 μmol/kg)	0.332±0.026	0.325±0.016	0.296±0.031
Na_2SeO_3 (2.5 μmol/kg)	0.388±0.040	0.352±0.017	0.314±0.028
Na_2SeO_3 (5.0 μmol/kg)	0.349±0.053	0.355±0.033	0.327±0.027
Na_2SeO_3 (10 μmol/kg)	0.367±0.034	0.464±0.109*	0.386±0.016*

Note. *P<0.05 *vs* control group.

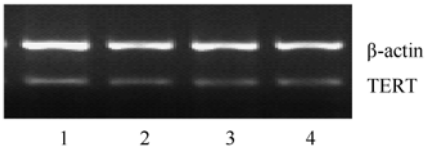

FIG. 1. Expression of TERT and β-actin mRNA in rat hepatocytes after exposure to selenium. RNA was isolated and analyzed by RT-PCR. Lanes 1-3: selenium-treated groups at the doses of 10, 5.0, and 2.5 μmol/kg; lane 4: control group.

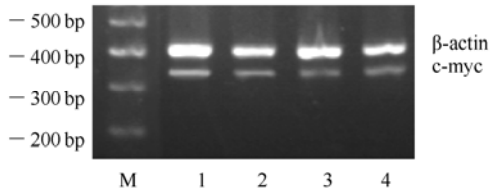

FIG. 2. Expression of c-myc and β-actin mRNA in rat hepatocytes after exposure to selenium. RNA was isolated and analyzed by RT-PCR. Lane M: marker; lanes 1-3: selenium-treated groups at the doses of 10, 5.0, and 2.5 μmol/kg; lane 4: control group.

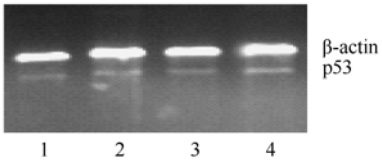

FIG. 3. Expression of p53 and β-actin mRNA in rat hepatocytes after exposure to selenium. RNA was isolated and analyzed by RT-PCR. Lane 1: control group; lanes 2-4: selenium-treated groups at the doses of 2.5, 5.0, and 10 μmol/kg.

TABLE 4

Effect of Selenium on c-myc and P53 Protein Expression in Rat Hepatocytes ($\bar{x} \pm s$, n=5)

Groups	c-myc	P53
Control (0 μmol/kg)	0.057±0.020	0.073±0.014
Na_2SeO_3 (2.5 μmol/kg)	0.063±0.026	0.074±0.013
Na_2SeO_3 (5.0 μmol/kg)	0.068±0.031	0.094±0.010*
Na_2SeO_3 (10 μmol/kg)	0.126±0.020*	0.101±0.011**

Note. *P<0.05, **P<0.01, *vs* Control.

DISCUSSION

One of the primary mechanisms underlying the cellular senescence is to control telomere shortening during cell division. Normal somatic cells express a low or even an undetectable telomerase level leading to a progressive loss of their telomeric sequences, thus initiating the process of senescence. Telomerase inactivation contributes to telomere loss during cellular senescence and aging[1]. Telomere shortening resulting from the absence of telomerase activity may constitute a factor in determining some age-related properties[7]. Ectopic expression of telomerase can prevent senescence of different cells and has a potential for intervention of the aging process based on tissue engineering, gene therapy or homografts[8]. Introduction of telomerase is proposed as a method to combat ageing via cell therapy and also as a possible approach to regenerate tissue[9]. A recent study has demonstrated that Ginkgo biloba extract can significantly increase telomerase activity and delay the onset of EPC senescence[10] and telomerase inactivation accelerates senescence of endothelial progenitor cells[11], indicating that telomerase inactivation and reactivation are closely related with the process of senescence and anti-senescence. Sodium selenite at nutritional doses could prolong the life span of hepatocytes L-02 by increasing the telomerase

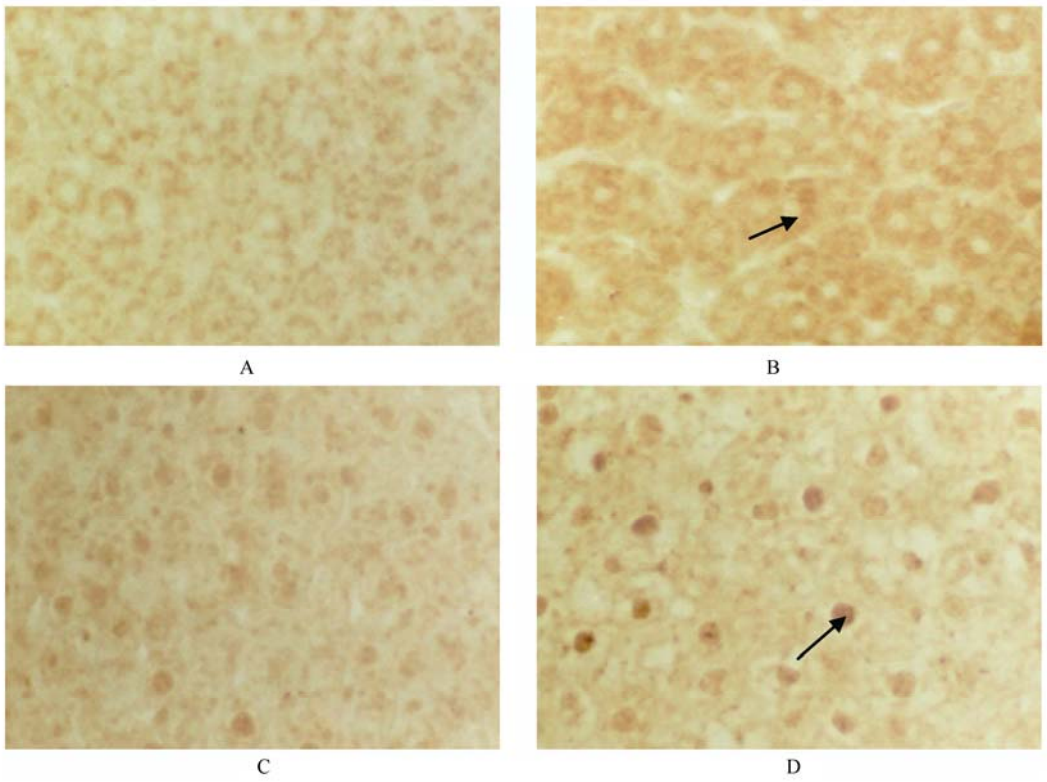

FIG. 4. Expression of c-myc and p53 in the control (A, C) and selenium treated groups (B, D) (×200).

activity and telomere length[5]. Several studies have indicated that selenium plays an important role in resisting senescence by regulating the telomere length and telomerase activity[2-3,5-6]. In our study, selenium increased the telomerase activity in a dose-dependent manner.

Telomerase complex, a specialized RNA template-containing reverse transcriptase, comprises telomerase RNA (TR) which is used as a template in DNA replication, TERT which is a telomerase catalytic subunit, and human telomerase-associated protein 1 (TEP1). Although TR contains the essential template region specifying the addition of telomerase sequence, its expression is not correlated with the telomerase activity. Up-regulation or down-regulation of TERT mRNA expression and telomerase activity during cell immortalization or differentiation was observed, suggesting that control of TERT expression at mRNA level mainly contributes to the regulation of telomerase enzymatic activity. Thus, TERT is the key component for the control of telomerase activity[12]. Selenium did not significantly increase the TERT mRNA expression at doses of 2.5, 5.0, and 10 μmol/kg, but significantly enhanced the TERT mRNA expression at the dose of 2.5 μmol/kg. Liu *et al.*[5] have reported that selenium at the dose of 0.5 or 2.5 μmol/L has anti-aging effects.

Cellular senescence and apoptosis represent two forms of genetically controlled growth arrest. Any escape from this restraint leads to oncogenic progression of cells. Apoptosis is a physiological process of cell death. It is also an important defense against cancer development. Attempts have been made to establish a relationship between telomerase activity and apoptotic pathways so as to develop novel strategies against cancer cell growth by co-targeting these pathways[13]. However, telomerase activity analyzed by TRAP assay in apoptotic cells remains unchanged as compared with the untreated control cells[13]. It has been shown that telomerase could protect rabbit lens epithelial cells against apoptosis[14]. While telomerase adds telomeric repeats *de novo* contributing to enhanced proliferative capacity and life span, it may also increase cellular survival by conferring resistance to apoptosis[15]. Ren *et al.*[16] reported that telomerase introduction and expression in normal human lung fibroblasts markedly increased the resistance to hydroxyl radical-induced apoptosis. When exposed to a lower concentration of rare earth elements, the telomerase activity of mononuclear cells from human peripheral

blood is higher, but the apoptotic rate of mononuclear cells is not affected[17]. In this research, telomerase activity was positively related to apoptosis of rat hepatocytes ($r=0.8876$, $P<0.05$), suggesting that the relationship between telomerase activity and apoptosis is complicated, and the detailed mechanisms need to be further clarified.

Oncogene-induced senescence is defined as irreversible cell cycle arrest of normal cells upon over-expression of oncogenes. The c-myc proto-oncogene encodes a transcription factor that plays a crucial role in various biological processes such as cellular proliferation, growth, apoptosis, metabolism, adhesion, protein synthesis, DNA replication, and angiogenesis. Myc inactivation is associated with several molecular features of cellular senescence. Wu *et al.*[18] have shown that Myc inactivation either *in vitro* or *in vivo* results in cellular senescence. Zhuang *et al.*[19] have suggested that one of the major functions of c-myc overexpression in melanoma progression is to continuously suppress senescence programs. The p53 tumor suppressor protein has an evident impact on the processes of cellular and organism aging. Aging phenotypes and pathways in organisms, like Saccharomyces cerevisiae, Caenorhabditis elegans, and Drosophila melanogaster, are regulated by p53. Modulating the expression of p53 might increase life span[20-21], indicating that c-myc and p53 over-expression may possibly inhibit senescence process. In our study, selenium at doses of 2.5, 5.0, and 10 μmol/kg increased the c-myc and p53 expression level in rat hepatocytes, indicating that selenium plays an important role in suppressing cellular senescence by regulating the expression of c-myc and p53.

One oncogene that might activate TERT in the natural context is c-myc. The Myc gene is frequently deregulated in human tumors and myc overexpression may cause telomerase reactivation and telomere stabilization, which, in turn, would allow permanent proliferation[22]. Myc induces telomerase both in normal human mammary epithelial cells (HMECs) and in normal human diploid fibroblasts. Myc increases expression of hEST2 (hTRT/TP2), a limiting subunit of telomerase, and both Myc and hEST2 can prolong the life span of HMECs[23]. The expression of hTERT and c-myc mRNA indicates that activation and up-regulation of hTERT might be conferred by overexpression of the c-myc gene[24]. hTERT mRNA regulation is not significantly associated with the c-myc level[25]. No correlation between hTERT and c-myc has been found in endometrial hyperplasia or carcinoma[26]. The p53 tumor suppressor controls cell growth and survival through transcriptional regulation of gene expression. It has been shown that human telomerase reverse transcriptase (hTERT) gene is down regulated by p53[27]. Beliveau A and Yaswen P[28] have reported that telomerase reduces the basal level of activated p53 and increases the cellular tolerance to other p53-dependent signals. In our study, c-myc was not closely related to TERT expression.

Selenium at relatively high doses induces apoptosis and over-expression of c-myc and p53, and also increases C-myc and P53 protein expression in rat hepatocytes. While c-myc over-expression induced by selenium is correlated with hepatocellular apoptosis[29], myc over-expression elevates intracellular ROS levels and results in oxidative DNA damage, as assessed by levels of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxo-dG)[30]. Rudolf *et al.*[31] have reported that selenite induces caspase-independent apoptosis in cervical carcinoma cells mostly by oxidative stress-mediated activation of p53 and p38 pathways. Over-expression of c-myc and p53 mediated by oxidative stress may represent a key factor for selenium-induced apoptosis. Selenium at relatively low doses can ameliorate apoptosis and oxidative stress[32].

In conclusion, selenium can slightly increase telomerase activity and TERT expression, but induces significant apoptosis and over-expression of c-myc and p53 at a relatively high dose. The beneficial effects of selenium on senescence and aging may be mediated by telomerase activation and expression of TERT, c-myc, and p53. Further study is needed to display the exact mechanism underlying the relationship between apoptosis and expression of TERT, c-myc and p53.

REFERENCES

1. Liu Y, Bohr V A, Lansdorp P (2008). Telomere, telomerase, and aging. *Mech Ageing Dev* **129**(1-2), 1-2.
2. Olivieri O, Stanzial A M, Girelli D, *et al*. (1994). Selenium status, fatty acids, vitamins A and E, and aging: the Nove Study. *Am J Clin Nutr* **60**(4), 510-517.
3. Liu Q, Wang H, Hu D, *et al*. (2004). Effects of trace elements on the telomere lengths of hepatocytes L-02 and hepatoma cells SMMC-7721. *Biol Trace Elem Res* **100**(3), 215-227.
4. Houben J M, Moonen H J, van Schooten F J, *et al*. (2008). Telomere length assessment: biomarker of chronic oxidative stress? *Free Radic Biol Med* **44**(3), 235-246.
5. Liu Q, Wang H, Hu D C, *et al*. (2003). Effects of sodium selenite on telomerase activity and telomere length. *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai)* **35**(12), 1117-1122.
6. Cui Q H, Tang C C, Huang Y G (2002). Effects of lead and selenium on telomere binding protein Rap1p, telomerase and telomeric DNA in Saccharomyces cerevisiae. *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai)* **34**(2), 240-244.
7. Hornsby P J (2007).Telomerase and the aging process. *Exp Gerontol* **42**(7), 575-581.
8. Ostler E L, Wallis C V, Aboalchamat B, *et al*. (2000). Telomerase and the cellular lifespan: implications of the aging process. *J Pediatr Endocrinol Metab* **13**(Suppl 6), 1467-1476.

9. Shawi M, Autexier C. (2008). Telomerase, senescence and ageing. *Mech Ageing Dev* **129**(1-2), 3-10.
10. Dong X X, Hui Z J, Xiang W X, *et al*. (2007). Ginkgo biloba extract reduces endothelial progenitor-cell senescence through augmentation of telomerase activity. *J Cardiovasc Pharmacol* **49**(2), 111-115.
11. Imanishi T, Kobayashi K, Kuki S, *et al*. (2006). Sirolimus accelerates senescence of endothelial progenitor cells through telomerase inactivation. *Atherosclerosis* **189**(2), 288-296.
12. Chen H J, Yu R A, He L F, *et al*. (2007). Inhibitory effects of selenium on telomerase activity and hTERT expression in cadmium-transformed 16HBE cells. *Biomed Environ Sci* **20**(4), 307-312.
13. Zhang J X, Zhang Z K, Sheng H P, *et al*. (2000). Telomerase activity is not related to apoptosis in leukemic cell lines. *Life Sci* **66**(18), 1713-1723.
14. Xiang H, Wang J, Mao Y W, *et al*. (2000). hTERT can function with rabbit telomerase RNA: regulation of gene expression and attenuation of apoptosis. *Biochem Biophys Res Commun* **278**(3), 503-510.
15. Bermudez Y, Erasso D, Johnson N C, *et al*. (2006). Telomerase confers resistance to caspase-mediated apoptosis. *Clin Interv Aging* **1**(2), 155-167.
16. Ren J G, Xia H L, Tian Y M, *et al*. (2001). Expression of telomerase inhibits hydroxyl radical-induced apoptosis in normal telomerase negative human lung fibroblasts. *FEBS Lett* **488**(3), 133-138.
17. Yu L, Dai Y, Yuan Z, *et al*. (2007).Effects of rare earth elements on telomerase activity and apoptosis of human peripheral blood mononuclear cells. *Biol Trace Elem Res* **116**(1), 53-59.
18. Wu C H, van Riggelen J, Yetil A, *et al*. (2007). Cellular senescence is an important mechanism of tumor regression upon c-Myc inactivation. *Proc Natl Acad Sci U S A* **104**(32), 13028-13033.
19. Zhuang D, Mannava S, Grachtchouk V, *et al*. (2008). C-MYC overexpression is required for continuous suppression of oncogene-induced senescence in melanoma cells. *Oncogene* **27**(52), 6623-6634.
20. Schmid G, Kramer M P, Maurer M, *et al*. (2007). Cellular and organismal ageing: Role of the p53 tumor suppressor protein in the induction of transient and terminal senescence. *J Cell Biochem* **101**(6), 1355-1369.
21. Wesierska-Gadek J, Ranftler C, Schmid G (2005). Physiological ageing: role of p53 and PARP-1 tumor suppressors in the regulation of terminal senescence. *J Physiol Pharmacol* **56**(Suppl 2), 77-88.
22. Cerni C (2000). Telomeres, telomerase, and myc. An update. *Mutat Res* **462**(1), 31-47.
23. Wang J, Xie L Y, Allan S, *et al*. (1998). Myc activates telomerase. *Genes Dev* **12**(12), 1769-1774.
24. Geng Z, Zhang D, Liu Y (2003). Expression of telomerase hTERT in human non-small cell lung cancer and its correlation with c-myc gene. *Chin Med J (Engl)* **116**(10), 1467-1470.
25. Liu Y C, Chen C J, Wu H S, *et al*. (2004). Telomerase and c-myc expression in hepatocellular carcinomas. *Eur J Surg Oncol* **30**(4), 384-390.
26. Dong Y, Li T, Wang Y, *et al*. (2004).Expression of human telomerase reverse transcriptase and c-myc transcripts in endometrial carcinoma and its precursors. *Zhonghua Bing Li Xue Za Zhi* **33**(1), 40-43. (In Chinese)
27. Rahman R, Latonen L, Wiman K G (2005). hTERT antagonizes p53-induced apoptosis independently of telomerase activity. *Oncogene* **24**(8), 1320-1327.
28. Beliveau A, Yaswen P (2007). Soothing the watchman: telomerase reduces the p53-dependent cellular stress response. *Cell Cycle* **6**(11), 1284-1287.
29. Yu R A, Yang C F, Chen X M (2006). DNA damage, apoptosis and C-myc, C-fos, and C-jun overexpression induced by selenium in rat hepatocytes. *Biomed Environ Sci* **19**(3), 197-204.
30 K C S, Cárcamo J M, Golde D W (2006). Antioxidants prevent oxidative DNA damage and cellular transformation elicited by the over-expression of c-MYC. *Mutat Res* **593**(1-2), 64-79.
31. Rudolf E, Rudolf K, Cervinka M (2008). Selenium activates p53 and p38 pathways and induces caspase-independent cell death in cervical cancer cells. *Cell Biol Toxicol* **24**(2), 123-141.
32. Yu R A, Xia T, Wang A G, *et al*. (2006). Effects of selenium and zinc on renal oxidative stress and apoptosis induced by fluoride in rats. *Biomed Environ Sci* **19**(6), 439-444.

(*Received October* 10, 2008 *Accepted May* 26, 2009)