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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.03.061.

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The Effect of Resveratrol on the Werner Syndrome RecQ Helicase Gene and Telomerase Activity

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Abstract: Various protein factors, including telomerase and WRN helicase, are involved in telomere maintenance. Resveratrol (Rsv), a polyphenol that extends the lifespan of diverse species is an activator of SIRT1, a NAD^+ dependent deacetylating enzyme in mammalian cells. Here, we examined the changes in gene expressions and promoter activities of WRN helicase and telomerase after Rsv treatment. This treatment increased the amount of *WRN* transcript and protein product by activating its promoter and telomerase promoter activity and gene expression. However cell proliferation was not changed. This suggests that Rsv induces telomere maintenance factors like WRN helicase without affecting cell proliferation.

Keywords: BLM, Resveratrol, Telomerase, Sp1, TERT, WRN.

INTRODUCTION

Aging or senescence is a complicated biological process and various factors are involved in its regulatory molecular mechanisms [1-3]. Telomeres, which are the ends of eukaryotic chromosomes, function to protect chromosome ends from fusion and degradation [4]. In human fibroblasts, telomeres are shortened by repeated cell divisions and their length correlates with cellular senescence [4,5]. Progeria syndrome patients are known to show premature aging that is different from normal process. Studies on the human progeroid diseases suggest that nuclear structure and DNA repair systems play important roles in the control of the aging process and life span [6]. Werner's syndrome (WS) patients show also premature aging accompanied with chromosomal instability by mutations on the *WRN* gene, which is involved in DNA repair and telomere maintenance [7]. These observations imply that aging of organisms is controlled by the nuclear functions especially chromosomal maintenance.

Alternatively, aging can be explained by a mitochondrial free radical theory [1]. Reactive oxygen species (ROS) are generated by mitochondrial respiration, and the highly reactive ROS damage inner cellular components including lipids, proteins, and DNA [8]. In some cases, caloric restriction (CR) could reduce ROS production and increases expression of ROS-metabolizing enzymes such as catalase and superoxide dismutases (SODs) [9]. CR extends the mean and maximum life spans of numerous organisms [10]. One of the molecular mechanisms induced by CR is the activation of sirtuins, a conserved family of NAD^+- dependent deacetylases [11]. Resveratrol (Rsv), which is a polyphenol contained in grape skins and red wine, can activate sirtuin-mediated deacetylation [12,13]. Although, it has been reported that Rsv has no obvious effect [14], Rsv could increase median and maximum life spans of yeast, nematode, and fruit flies [12]. In addition, Rsv was reported to improve survival of high-calorie diet mice [15], also suggesting that this compound is a CR mimetic drug. The molecular mechanism of the extension of life span caused by CR is thought to be similar to that caused by Rsv. In order to establish a treatment for aging cells to keep a potency to proliferate and differentiate, it is required to investigate cellular signals induced by Rsv.

In this study, we show that Rsv increases *WRN* promoter activity, and that its gene and protein expressions are accompanied by up-regulation of telomerase in HeLa S3 cells. Moreover, the viability of the cells was not influenced by the treatment for 24 h. These observations suggest that Rsv is an activator of telomerase and WRN without affecting cell death signals.

MATERIALS AND METHODS

Cell Culture

Human cervical carcinoma (HeLa S3) cells were grown in Dulbecco's modified Eagle's (DME) medium (Nacarai, Tokyo, Japan), supplemented with 10% fetal bovine serum (FBS) (Sanko-Pure Chemical, Tokyo, Japan) and penicillin-streptomycin at 37 °C in a humidified atmosphere with 5% CO_2.

Cell Viability Assay (MTS Assay)

An MTS assay was performed as described in the product manual. In brief, cells were cultured in microtiter plate wells with 20 μl of MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2(4-sulfophenyl)-2H-tetrazolium] solution (Promega, Madison, WI) per well (containing 100 μl

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of cell culture) and incubated for 3 h in a 37 °C, 5% CO_2 humidified incubator. Absorbance at 570 nm was measured by microtiter plate reader (Thermo electron Corp, Vantaa, Finland) and normalized by absorbance at 630 nm.

Construction of Luc-Reporter Plasmids

Luciferase (Luc) reporter plasmids carrying the human *WRN*, *BLM*, *HELB*, *TERT*, and *p21* promoter regions were designated pGL4-BLM, pGL4-HELB, pGL4-WRN, pGL4-TERT, and pGL4-p21 [16]. The Luc reporter plasmid that contains 5'-flanking region of the human Pif1 gene was named pGL4-Pif1 [17].

Transient Transfection and Luc Assays

Plasmid DNAs were transfected into HeLa S3 cells by the DEAE-dextran method [16,18]. After 6 to 24 h of transfection, *trans*-Resveratrol (Cayman Chem., Ann Arbor, MI) was added to the culture medium. After a further 16 to 40 h of incubation, cells were collected and lysed with 100 µl of 1 x Cell culture lysis reagent, mixed, and centrifuged at 12,000 x g for 5 sec. The supernatant was stored at – 80 °C. The Luc assay was performed with a Luciferase assay system (Promega), as described previously [16,18].

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

RT-PCR was carried out as described previously [16,18]. First-strand cDNAs were synthesized with ReverTra Ace (Toyobo, Tokyo, Japan), random primers (Takara), and total RNAs extracted from HeLa S3 cells. Primer pairs to amplify human *BLM*, *HELB*, *WRN*, *Sp1*, and *β-actin* cDNAs have been reported previously [16], and the primers for amplifying *SIRT1* cDNAs were hSIRT1-468; 5'- GCGATTGGGTA CCGAGATAAC -3' and hASIRT1-652; 5'- GTTCGAG-GATCTGTGCCAATC -3'. Conditions for the PCR were as follows: 94 °C 30 sec, 55 °C 30 sec, and 72 °C 1 min, with 27 (*BLM*), 26 (*WRN* and *HELB*), 22 (*Sp1*), 22 (*SIRT1*), and 20 (*β -actin*) cycles. PCR products were electrophoresed on 5% acrylamide gels and stained with ethidium bromide.

Quantitative Real-time PCR

Real time PCR analysis was carried out using the Mx3000P Real-Time QPCR System (Stratagene, La Jolla, CA) as described previously [16]. For PCR amplification, cDNAs were amplified using SYBR Green Realtime PCR Master Mix (Toyobo) and 0.3 µM of each primer pair. The primer pairs for amplifying *GAPDH* cDNA were hGAPDH556; 5'- TGCACCACCAACTGCTTAGC-3' and hGAPDH642; 5'- GGCATGGACTGTGGTCATGAG-3'. Amplification was carried out initially for 30 sec at 94 °C, followed by 40 cycles (94 °C 30 sec, 55 °C 30 sec, and 72°C 1 min). Quantitative PCR analysis for each sample was carried out in triplicates. Relative gene expression values were obtained by normalizing C_T (threshold cycle) values of target genes in comparison with C_T values of the *GAPDH* gene using the $\Delta\Delta C_T$ method.

Western Blot Analysis

Western blot analysis was carried out as previously described [16], with antibodies against WRN, and Sp1 (Santa Cruz Biothchnology, Santa Cruz, CA) followed by addition of horseradish peroxidase (HRP)-conjugated secondary antibody (Calbiochem, Darmstadt, Germany). Signal intensities were quantified with an LAS4000 system and MultiGauge Software (Fuji Film, Tokyo, Japan).

Telomerase Amplification Protocol (TRAP) Assay

Cell pellets ($3x10^5$) were treated with 200 µl of ice-cold 1 x CHAPS lysis buffer (10 mM Tris-HCl [pH 7.5], 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM Benzamidine, 5 mM β-mercaptoethanol, 0.5% CHAPS, and 10% Glycerol), incubated on ice for 30 min, and centrifuged at 12,000 x g for 20 min at 4 °C. The supernatant was then stored at –80 °C. TRAP assays were performed with a TRAP assay kit (Chemicon, Temecula, CA) as described previously [16]. Signal intensities of the DNA ladders and internal controls were quantified with MultiGauge Software.

RESULTS

Proliferation of HeLa S3 Cells After Rsv Treatment

As shown in Fig. (**1**), the proliferation of HeLa S3 cells was not suppressed after 24 h treatment with 40 µM of Rsv measured by MTS. This suggests that Rsv does not induce cell-damaging signals in these experimental conditions.

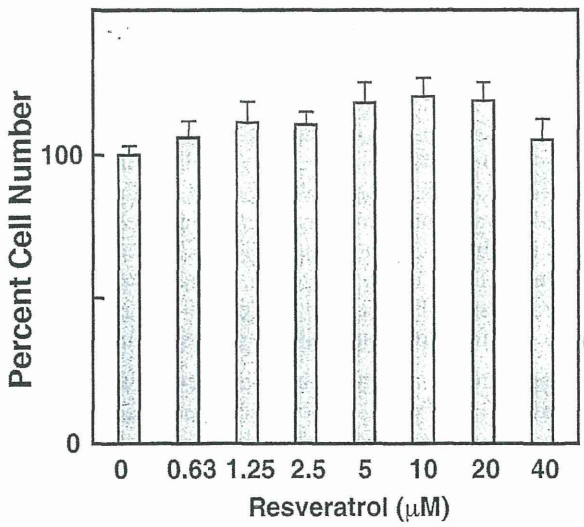

Fig. (1). Resveratrol (Rsv) does not affect proliferation of HeLa S3 cells. HeLa S3 cells were treated with Rsv (1-40 µM) for 24 h. Results represent cell viabilities measured by the MTS assay. The results show the mean ± SD of eight independent experiments.

Augmentation of Expression of DNA Helicase-encoding Genes by Rsv

Quantitative real time PCR showed that the expression of the *WRN*, *HELB*, and *Sp1* genes increased by the increase of the concentrations of Rsv up to 10 µM in the culture medium for 24 h (Fig. **2B**). On the other hand, *BLM* gene expression

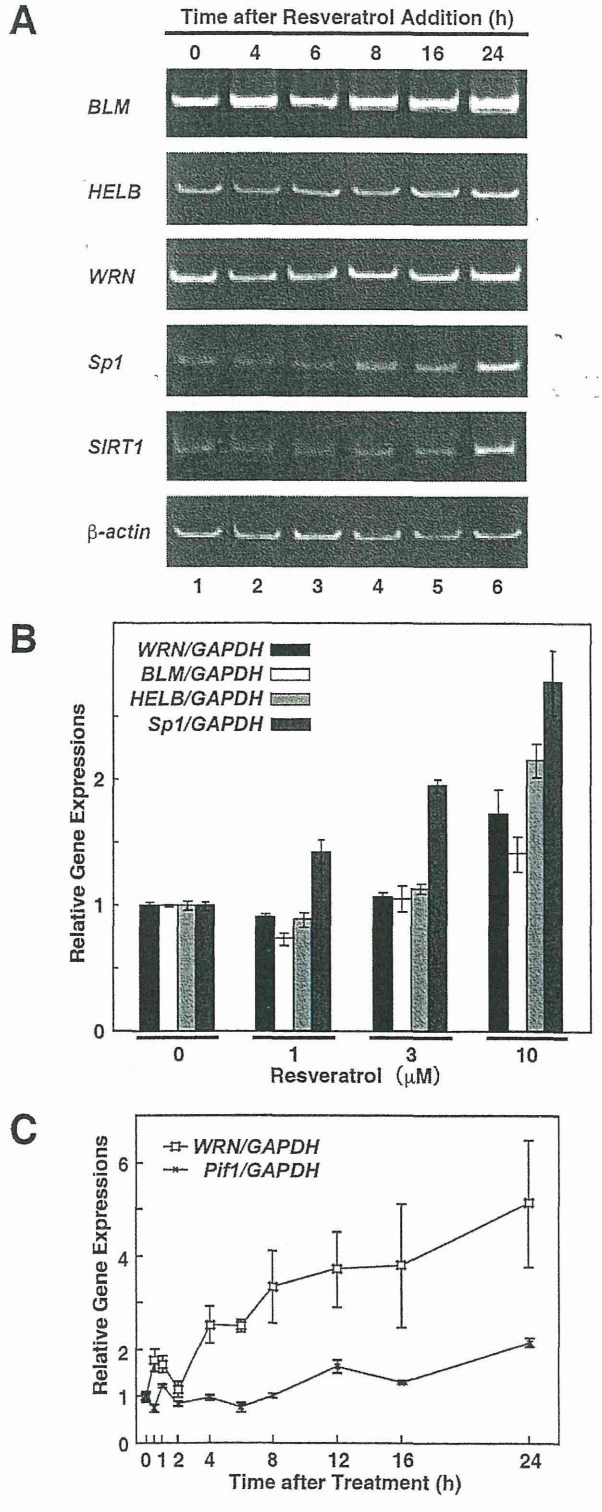

Fig. (2). Changes in gene expressions of various genes after Rsv treatment. (**A**) HeLa S3 cells were treated with 10 μM Rsv for 0, 4, 6, 8, 16, and 24 h (lanes 1, 2, 3, 4, 5 and 6, respectively). Total RNAs were extracted from the cells, and synthesized cDNAs were subjected to PCR with appropriate primer pairs to amplify *BLM*, *HELB*, *WRN*, *Sp1*, *SIRT1*, and *β -actin* cDNA. (**B**) HeLa S3 cells were treated with different concentrations of Rsv (0 to 10 μM) for 24 h. Realtime PCR analyses were performed with cDNA prepared from similar experimental conditions as described above. Results show relative gene expressions of *WRN*, *BLM*, *HELB*, and *Sp1* compared with that of *GAPDH*. The results show the mean ± SD of three independent assays. (**C**) Realtime RT-PCR was carried out to analyze *WRN* and *Pif1* gene expression in HeLa S3 cells after 10 μM of Rsv treatment for 0 to 24 h. The results show relative *WRN/GAPDH*, and *Pif1/GAPDH* gene expression ratios compared with those of Rsv non-treated cells. The values are the mean ± SD of four independent experiments.

was not greatly changed. Next, we examined whether the amounts of *WRN* transcripts were accumulated in a time-dependent manner. As shown in Fig. (**2C**), the relative amount of *WRN* transcripts was greater than those of the *Pif1* transcripts after 4-24 h treatment of 10 μM of Rsv.

Effect of Rsv on the WRN Promoter

To test whether human *WRN*, *BLM*, *TERT*, *p21*, and *HELB* promoters are affected by Rsv treatment, transient transfection experiments were carried out (Fig. **3A**). Luc activities of pGL4-WRN, -BLM, -HELB, -TERT, and -p21 transfected cells were normalized to that of the pGL4-Pif1 transfected cells, because Pif1 has been suggested to have a negative effect on telomere elongation [19]. As shown in Fig. (**3A**), relative Luc activities of these reporter-vector transfected cells were augmented by 10 μM Rsv.

To compare time-dependent responses of the promoters precisely, HeLa S3 cells were treated with Rsv after 6 h of transfection and collected after further incubation from 0 to 40 h. Increases in the *WRN/Pif1* and *TERT/Pif1* promoter activity ratios were observed after 24 h of treatment (Fig. **3B**). Next, Rsv was added after 24 h of transfection, and the cells were harvested after further 0 to 48 h incubation (Fig. **3C**). In these experimental conditions, the magnitude of fold activation of the pGL4-WRN transfected cells after 24 to 48 h Rsv treatment was greater than that of the pGL4-Pif1 transfected cells. Taken together, these results indicate that Rsv up-regulates the *WRN* and *TERT* promoters in HeLa S3 cells.

Expression of WRN Protein After Rsv Treatment

The data above indicate that the *WRN* promoter responds to Rsv and that transcripts are accumulated in the cells. To examine *WRN* protein expression, Western blotting with samples from cells treated with 10 μM Rsv was performed. WRN protein expression increased more than three fold compared with the control level after 16 h Rsv treatment (Fig. **4**). Surprisingly, the amount of WRN protein in 24 h Rsv-treated cells decreased, accompanied by a decrease in Sp1 protein expression (Fig. **4B**). Because *WRN* gene expression increased continuously after 24 h Rsv treatment (Fig. **2**), WRN protein might be down-regulated or degraded at the post-translational level.

Activation of Telomerase by Rsv Treatment

Because similar responses in *WRN* promoter and gene/protein expressions were observed after Rsv, as in the

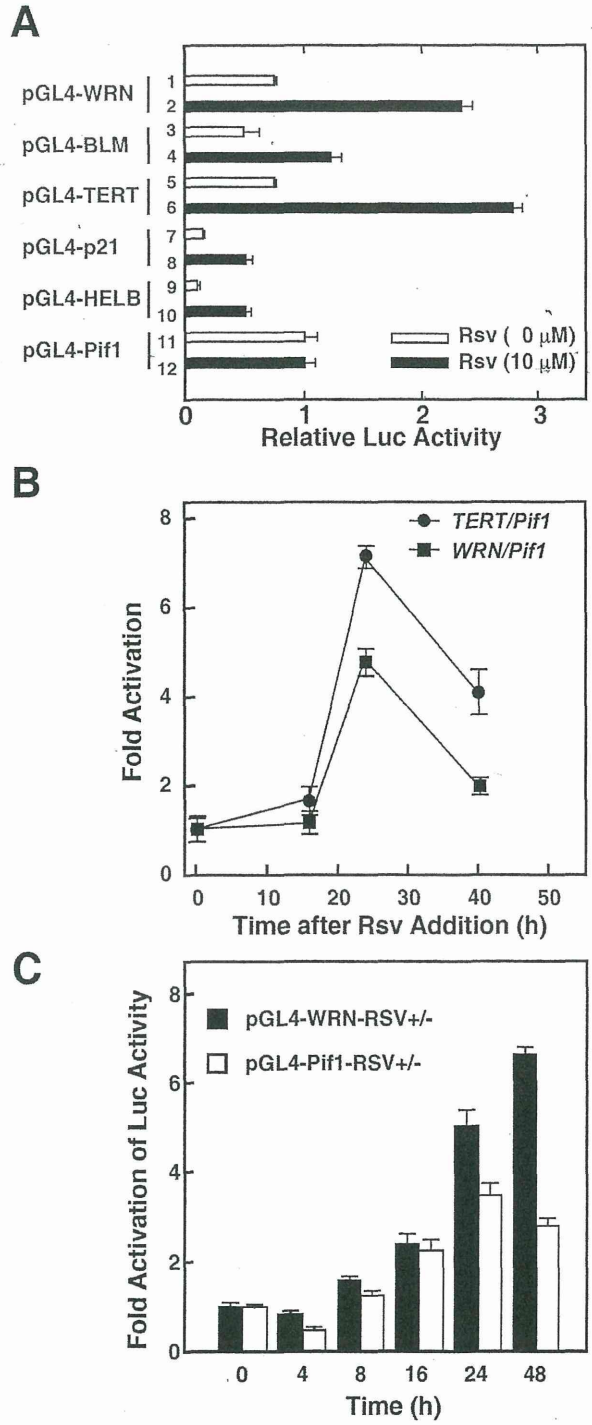

Fig. (3). Effect of Rsv on *WRN*, and *TERT* promoter activities. (**A**) HeLa S3 cells were transiently transfected with pGL4-WRN (columns 1 and 2), pGL4-BLM (columns 3 and 4), pGL4-hTERT (columns 5 and 6), pGL4-p21 (columns 7 and 8), pGL4-HELB (columns 9 and 10), and pGL4-Pif1 (columns 11 and 12). After 6 h incubation, cells were treated with 10 μM Rsv for a further 16 h then Luc samples were prepared. The results show relative Luc activities of these reporter plasmid-transfected cells compared with that of pGL4-Pif1 vector transfected cells. The values are the mean ± SD of four independent assays. (**B**) Similar experiment described in the legend to (**A**) was performed. Fold activation indicates the WRN/Pif1 and TERT/Pif1 promoter activity ratios of Rsv treated cells compared with those of Rsv non-treated cells. The results show the mean ± SD of three independent assays. (**C**) HeLa S3 cells were treated with Rsv after 24 h of transfection. After further 24 h incubation, cells were harvested and Luc assays were performed. The results show relative Luc activities of these reporter plasmid-transfected cells compared with that of Rsv non-treated cells. The values are the mean ± SD of three independent assays.

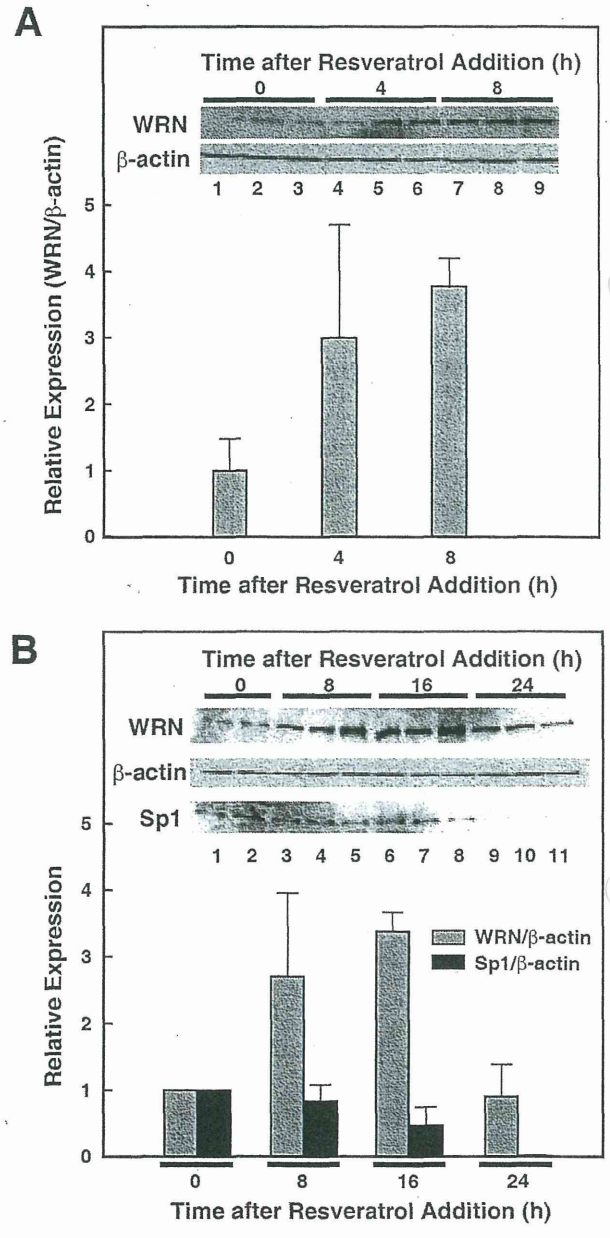

Fig. (4). Rsv treatment-induced amount of WRN in whole protein extracts from HeLa S3 cells. (**A**) HeLa S3 cells (1.0×10^5) were treated with Rsv (10 μM) for 0, 4, and 8 h. Proteins extracted from Rsv-treated cells were separated by a 7.5% SDS-PAGE, and Western blotting was performed with anti-WRN and anti-β-actin antibodies (upper and lower panels, respectively). Then, each band was quantified and results show relative WRN/β-actin protein expression ratio compared to that of the Rsv non-treated cells. (**B**) HeLa

S3 cells were treated with 10 μM Rsv for 0, 8, 16 and 24 h and Western blotting was performed with anti-WRN, anti-β-actin, and anti-Sp1 antibodies. The signal intensity of each band was quantified and the results show relative protein levels compared with that of Rsv-non-treated cells.

case of 2DG treatment [16], we speculated that telomerase might be activated by Rsv. As confirmation, a TRAP assay was performed with the protein samples from HeLa S3 cells treated with 10 μM Rsv for 8 to 24 h (Fig. **5A**). The results showed that telomerase activity reached its highest point at 16 h treatment and decreased after a further 8 h (Fig. **5B**). The pattern of changes of telomerase after Rsv treatment is similar to that of WRN.

DISCUSSION

Genes suggested as controlling the aging process encode proteins involved in DNA-damage responses, DNA-repair synthesis, telomere maintenance, insulin signaling, ROS metabolism, and construction of nuclear membranes [1,3,6]. In other words, the aging process could be affected by alteration of variety of gene expression induced by epigenetic and environmental changes. On the other hand, it seems that the life spans are pre-set or pre-determined by the genome of the organism because the maximum life spans of species are almost constant. From a genetic study of *C. elegans,* it has been suggested that the insulin-mediated signal transduction system plays an important role in controlling life span [20, 21].

CR or reduced caloric intake is the only conclusive and reproducible intervention that can slow aging and maintain health in mammals [22]. Given that insulin enhances cellular glucose intake, CR might have the opposite effect of insulin signaling. Previously, we indicated that 2DG, a potent inhibitor of glucose metabolism, up-regulated the *WRN* gene and protein expressions accompanied by activation of telomerase in HeLa S3 cells [16]. Recent studies have suggested that Rsv also has positive [23] and negative [24] effects on telomerase activity. The effect of Rsv on telomerase might be dependent on whether the experimental conditions induce apoptosis or not. Recently, it was reported that Rsv activates an ATM/ATR-dependent DNA damage response [25]. Therefore, if apoptosis was not induced, Rsv would up-regulate chromosomal DNA maintenance factors including telomerase. In this study, we observed the positive effect of Rsv on *WRN* gene expression and telomerase activity without growth inhibition for 24 h. Rsv is known to be a CR mi-

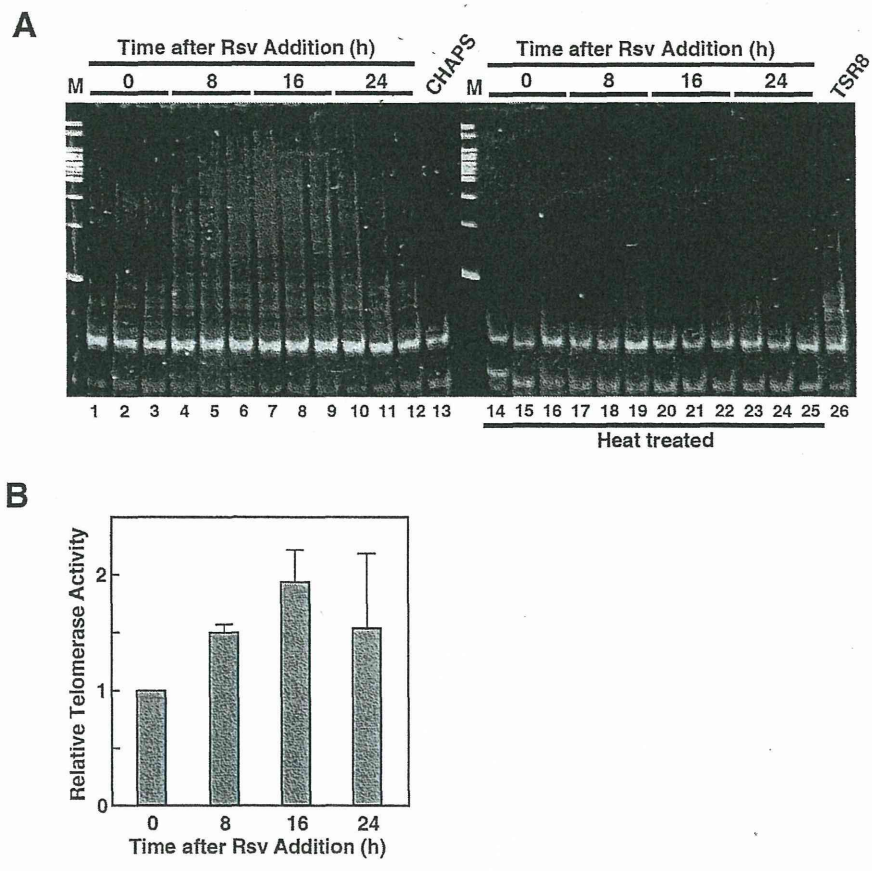

Fig. (5). Rsv treatment induces telomerase activity in HeLa S3 cells. (**A**) CHAPS lysis buffer extracts (20 ng) from HeLa S3 cells were analyzed by the TRAP assay. HeLa S3 cells were treated with 10 μM Rsv for 0 (lanes 1-3), 8 (lanes 4-6), 16 (lanes 7-9), and 24 h (lanes 10-12). Lanes 14 to 25 represent backgrounds with samples that were incubated at 85°C for 10 min. 1 x CHAPS (lane 13) and TSR8 (lanes 26) represent negative and positive controls, respectively. Lane M represents a 100-bp ladder marker. (**B**) Signal intensities of TRAP-products and internal controls were quantified and telomerase activities were calculated as described in the manufacturer's protocol. Histograms show relative telomerase activities compared with Rsv non-treated cells. Results show the mean ± SD of three independent assays.

metic drug that activates sirtuin (SIRT1)-mediated deacetylation [12] and the activation of SIRT1 by Rsv treatment has been reported to improve mitochondrial function [26]. These observations suggest that common signal molecules, such as FoxO [27], might have been affected by 2DG or Rsv to up-regulate promoter activities of the *WRN* and *TERT* genes. In addition, NF-κB activity has been reported to be inhibited by Rsv [28]. Therefore, NF-κB might be one of the candidates for the regulation of the *WRN* and *TERT* promoters. From comparison of the DNA sequence motifs of the *WRN* and *TERT* promoters, multiple GC-boxes or Sp1 binding sequences are commonly found in both promoter regions [16], suggesting that GC-box binding factors are affected by CR mimetic compounds to co-regulate telomere maintenance factors. Because the Sp1 protein decreased after Rsv treatment (Fig. **4B**), other GC-box associating factors might be involved in the positive regulation of the *WRN* and *TERT* promoters. However, these possibilities are yet to be elucidated.

2DG, as a CR mimetic drug, has a very narrow therapeutic range bordering on toxicity, making it difficult to be used as a drug candidate for human beings [22]. On the other hand, Rsv activates SIRT1 but it could induce cell-death or apoptosis [24]. Further analyses are needed to indicate the transcription factor(s) that are responsible for the up-regulation of *WRN* and *TERT* gene expressions by these CR mimetic compounds. Introduction of the expression vectors of these transcription factors with siRNAs for cDNAs of glucose metabolizing enzymes into cells might protect chromosomes and telomeres, thereby elongating life span without toxic effects.

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ABBREVIATIONS

BS	=	Bloom's syndrome
CR	=	Caloric restriction
2DG	=	2-deoxy-D-glucose
FCS	=	Fetal calf serum
HELB	=	Helicase B
HGPS	=	Hutchinson-Gilford progeria syndrome
Luc	=	Luciferase
MTS	=	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2(4-sulfophenyl)-2H-tetrazolium; inner salt
ROS	=	Reactive oxygen species
Rsv	=	Resveratrol
RT-PCR	=	Reverse transcriptase polymerase chain reaction
SDS-PAGE	=	SDS-polyacrylamide gel electrophoresis
SOD	=	Superoxide dismutase
TBS	=	TRIS buffered saline
TRAP	=	Telomerase amplification protocol
WS	=	Werner's syndrome.

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シンポジウム：摂食障害の新たな展開

脂質代謝の変化からみたカロリー制限による抗老化・寿命延長作用のメカニズム

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抄録：カロリー制限（caloric restriction：CR）は，老化過程を抑制，加齢に伴う疾患の発生を遅延し，平均および最大寿命を延長する唯一の簡便な再現性の高い方法として，老化研究に広く応用されている．しかし，そのメカニズムはいまだ解明されていない．一般にCRは，成長を抑制し，身体を小さく保ち，脂肪組織量を減少させ，高血糖および高インスリン血症を抑制，炎症を抑制，低体温で，脂質やエネルギー代謝を修飾し，内因性および外因性ストレスに対する抵抗性を増強，ミトコンドリア・バイオジェネーシスを亢進，サーチュインを活性化することが知られている．われわれは，CR動物では，食餌不足に対する適応反応として脂肪組織のリモデリングを介して脂質を効率的に利用していること，このような代謝の変化に脂肪酸合成関連遺伝子群発現の主要転写因子であるsterol regulatory element binding protein 1c（SREBP1c）が重要である可能性を示した．SREBP1cを介した*de novo*脂肪酸合成系の活性化がCRの主要なメカニズムの1つと考えられる．

Key words：カロリー制限，老化，脂質代謝，脂肪組織リモデリング，SREBP1c

カロリー制限の抗老化・寿命延長効果とは

1935年，米国のMcCayらは，離乳直後からの摂取カロリーの制限（caloric restriction：CR）により，ラットの寿命が延長することを報告した．それ以来75年以上にわたり，CRは，食餌制限（dietary restriction，food restriction），エネルギー制限（energy restriction）とも呼ばれ，唯一で簡便な再現性の高い寿命延長法として広く老化研究に応用されてきた[1)2)]．CRによる寿命延長効果は，酵母や線虫といった下等生物からげっ歯類にいたるまで広く観察されることから，進化の過程で保存されたメカニズムが関与することが示唆される．1989年Holliday[3)]は，CRによる抗老化・寿命延長作用のメカニズムとして，以下のように適応反応仮説を提唱し，食餌不足に対する神経内分泌および代謝の変化の重要性を進化論的観点から説明した．食餌が豊富な時期には，個体は成長し，強い大きな個体で積極的に生殖することで子孫を増やし，さらに過剰なエネルギーを脂肪組織に貯蔵する．一方，食餌が不足する時期には，個体の成長や生殖を抑制し，脂肪組織に貯えたエネルギーを使いながら，寿命を延長し，食餌が十分に得られる時期を待つ．このような食餌不足に対する適応能力の発達した動物が，進化の過程で選択されてきた．CRは，この食餌不足に対する適応反応を活性化し，抗老化・寿命延長をもたらすのではないかと考えられる．

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ほ乳類では，げっ歯類を中心に研究され，CRはさまざまな生理的加齢現象を抑制，老化に伴って発症する種々の疾患発症を遅延もしくは抑制し，寿命を延長することが明らかとなってきた．CRは活動性を維持し，平均および最大寿命を延長すること，CRの効果は，その期間や程度に比例すること，またエネルギー摂取の抑制にのみ依存しており，エネルギー制限のない各栄養素の摂取制限（糖質，脂質制限，タンパク質の摂取制限など）では，効果は得られないことが報告されている[1)2)]．一般的に，CR動物では，高血糖や高インスリン血症の抑制，酸化ストレスを含む内因性ストレスおよび外因性環境ストレスに対する抵抗性の増強，炎症の抑制，低体温，エネルギー代謝の効率化，ミトコンドリア・バイオジェネシスの活性化，サーチュインの活性化などが観察されており，これらがCRによる抗老化・寿命延長効果に重要であろうと考えられている．しかしながら，その詳細なメカニズムはいまだ解明されていない[4)〜6)]．

米国では1980年代の後半から数施設において，霊長類においてもCRの効果が有効であるか，サルを用いて検証されている．サルの寿命が長いことから最終的な結論は出ていないが，げっ歯類で観察されるCR動物の表現型はサルにおいても観察されることなどから，CRは霊長類においても有用であろうと考えられている[7)8)]．また，CRされたサルで観察された低体温，低血糖および年齢に伴って低下するdehydroepiandrosterone（DHEAS）の減少率が低いヒトの集団は，そうでない集団に比べて平均余命が長いことも報告されている[9)]．それゆえ，CRの有益な効果は，げっ歯類のみならず，ヒトを含む霊長類においても有効であろうと考えられる．

単一遺伝子の変異により長寿命を示すマウスやラット

CR以外寿命を延長する方法がなかったが，米国のBartkeら[10)]は1996年，Ames矮小マウスが長寿を示すと報告した．Ames矮小マウスは下垂体の発生や分化にかかわる転写因子であるProp1遺伝子に変異があり，成長ホルモンなどの下垂体前葉ホルモンの分泌に障害がある．Prop1遺伝子と類似した機能を有するPit1遺伝子に変異のあるSnell矮小マウス，次いで成長ホルモン放出ホルモン受容体遺伝子に変異のあるLittleマウスが長寿であることも報告された．その後，分子生物学および分子遺伝学の進歩と相まって，成長ホルモン受容体遺伝子やインスリン様成長因子1受容体遺伝子をノックアウトしたマウスなどが次々に作られ，筆者の知る限り現在まで報告されている単一遺伝子の改変により長寿を示すマウスやラットは約20種以上に及ぶ[11)]．これらを分類すると，その半数は成長ホルモン（GH）/インスリン様成長因子1（IGF-1）シグナルに関連する遺伝子を修飾したものであり，酸化ストレス/レドックス制御に関連するもの，脂肪細胞もしくはアディポサイトカインに関連するもの，およびその他の4つに大別できる．

われわれも，アンチセンス成長ホルモン遺伝子を成長ホルモン産生細胞に発現するようなトランスジェニック（tg）ラットにおいて，寿命を検討した．するとホモラット（tg/tg）の寿命はかえって短縮したが，ヘテロ（tg/−）ラットでは平均および最大寿命とも5〜10%延長した[12)]．CRにおいてもGH/IGF-1シグナルは抑制されるため，CRの有益な作用はGH/IGF-1シグナルの抑制に関連すると示唆された．そこで，野生型ラットと寿命が延長した（tg/−）ラットにCRを行った．すると予想に反して，野生型および（tg/−）ラットとも平均および最大寿命が同程度延長した[13)]．このことは，GH/IGF-1シグナルの抑制はCRの主要なメカニズムでないことを示唆している．