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Effects of Thujaplicins on the Promoter Activities of the Human *SIRT1* and Telomere Maintenance Factor Encoding Genes

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Abstract

Resveratrol (Rsv) has been shown to extend the lifespan of diverse range of species to activate sirtuin (SIRT) family proteins, which belong to the class III NAD^+ dependent histone de-acetylases (HDACs).The protein de-acetylating enzyme SIRT1 has been implicated in the regulation of cellular senescence and aging processes in mammalian cells. However, higher concentrations of this natural compound cause cell death. Therefore, novel compounds that have reduced cellular toxicity will be required for anti-aging therapy, especially for dermatological treatments. In this study, the Luciferase (Luc) expression vector pGL4-SIRT1 containing 396-bp of the 5'-upstream region of the human *SIRT1* gene was transfected into HeLa S3 cells and Luc assay was performed. The results showed that treatments with the natural compound, α-, β- and γ-thujaplicins increase the *SIRT1* promoter activity more than that with Rsv. Moreover, we carried out multiple transfection of Luc reporter vectors containing 5'-upstream regions of various human telomere maintenance factor encoding genes, and observed that β−thujaplicin (hinokitiol) activates *TERT*, *RTEL*, *TRF1*, *DKC1*, *RAP1 (TERF2IP)* and *TPP1(ACD)* promoters. These results suggest that that the β−thujaplicin could be used as anti-aging drugs to delay cellular senescence through activating *SIRT1* transcription along with strengthening stability of telomeres.

Keywords: Aging; Cellular senescence; Resveratrol; Shelterins; SIRT1; Telomere; Telomerase; Thujaplicin

Introduction

A natural polyphenolic compound Resveratrol (Rsv), which is known as a stimulator of NAD^+-dependent deacetylases sirtuin (SIRT) family proteins, sirtuin, elongates lifespan of model animals [1-5]. Previously, we reported that Rsv moderately activates the human *SIRT1* and *TERT* promoters inducing telomerase activity in HeLa-S3 cells [6,7]. Moreover, multiple transfection assays showed that promoter activities of the genes encoding human telomere maintenance factors (shelterin proteins) [8] are up-regulated by Rsv treatment [9], suggesting that natural polyphenol compounds, such as Rsv may affect chromosomal stabilities. Thus, Rsv and its related polyphenols are expected to become candidate drugs for anti-aging therapeutics. However, it should be noted that Rsv has cytotoxic effects by inducing apoptotic cell death, especially when it is used at higher doses [10-12]. Thus, in order to develop safe drugs with anti-aging effects, searching for alternative natural compounds that up-regulate *SIRT1* and shelterin gene expression and elucidation of their induction mechanisms are required.

β-Thujaplicin, which is also known as hinokitiol, is a tropolone derivative found in the heartwood of cupressaceous plants [13]. It has been reported to have a variety of biological effects, including induction of apoptosis [14] and differentiation [15], and anti-inflammatory [16], anti-bacterial [17] and anti-fungal [18] effects. In this study, we examined the effects of thujaplicins on the promoter activities of the human *SIRT1* and shelterin-encoding genes by multiple transient transfection and Luc reporter assay. Here, we show the up-regulating effects of three types of thujaplicins (α, β and γ) on the promoter activities of the human *SIRT1* and shelterin-encoding genes by multiple transient transfection and Luc reporter assay. Here, we show that β-thujaplicin (hinokitiol) is able to up-regulate these promoter activities. Furthermore, we propose that β-thujaplicin could be used as one of lead-compounds for developing anti-aging drugs.

Materials and Methods

Materials

trans-Resveratrol (Rsv) was purchased from Cayman Chem. (Ann Arbor, MI) [6,7]. α−, β- and γ-thujaplicins were purchased from Osaka Chemical Industry Ltd. (Osaka, Japan) [19]. Structures of these compounds are shown in (Figure 1).

Cell culture

Human cervical carcinoma (HeLa S3) cells [20] were grown in Dulbecco's modified Eagle's (DME) medium (WAKO Pure Chemical, Tokyo, Japan), supplemented with 10% fetal bovine serum (FBS)

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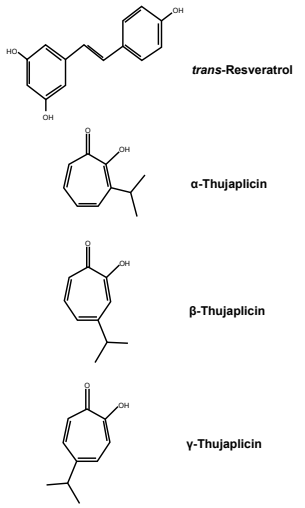

Figure 1: The structures of trans-Resveratrol and thujaplicins.

(Sanko Pure Chemical, Tokyo, Japan) and penicillin-streptomycin at 37°C in a humidified atmosphere with 5% CO_2.

Construction of Luc reporter plasmids

The Luc reporter plasmid pGL4-SIRT1 carrying 396-bp of the human *SIRT1* promoter region was constructed as described previously [7]. Other Luc reporter plasmids, which contain 300 to 500-bp of 5'-upstream regions of the human *PIF1*, *RTEL*, *TRF1*, *TRF2*, *TERT*, *TERC*, *TANK1*, *DKC1*, *TIN2*, *POT1*, *RAP1(TERF2IP)* and *TPP1(ACD)* genes, were constructed as described previously [9,21].

Transient transfection and Luc assay

Plasmid DNAs were transfected into HeLa S3 cells by the DEAE-dextran method [20-22]. The DNA transfected cells were divided into at least four dishes. After 24 h of transfection, Rsv or thujaplicins were added to the culture medium. After a further 24 h of incubation, cells were collected and lysed with 100 μL of 1 X cell culture lysis reagent, containing 25 mM Tris-phospate (pH 7.8), 2 mM DTT, 2 mM 1,2-diaminocyclohexane-N,N,N',N',-tetraacetic acid, 10% glycerol and 1% Triton X-100, then mixed and centrifuged at 12,000 × g for 5 sec. The supernatant was stored at -80°C. The Luc assay was performed with a Luciferase assay system (Promega) and relative Luc activities were calculated as described previously [20-22]. Multiple transfection of human shelterin promoter-containing Luc reporter plasmids with 96-well culture plate was performed as described previously [8,21].

Results

Effects of thujaplicins on the human *SIRT1* promoter

To examine whether the human *SIRT1* promoter is affected by α–, β– and γ–thujaplicins [19], transient transfection and Luc assays were carried out. Luc activities of pGL4-SIRT1 transfected cells were normalized to that of non-treated control cells. As shown in Figure 2A, the relative Luc activity of pGL4-SIRT1-transfected cells was prominently augmented by the addition of Rsv (10 μM) or α–, β– and γ-thujaplicins (10 μM) to the culture medium.

To examine the dose-dependent response to β-thujaplicin (hinokitiol), HeLa S3 cells were treated with 0 to 100 μM of β-thujaplicin after 24 h of transfection and collected after further 24 h incubation (Figure 2B). The half maximal effective concentration (EC_{50}) was estimated as 3.1 μM. These results indicate that 10 μM of μ-thujaplicin is enough to induce *SIRT1* promoter activity equal to Rsv (10 μM) treatment.

Effect of β–thujaplicin on the 5'-upstream regions of human genes encoding telomere maintenance factors

Multiple transcription experiments were carried out with various Luc reporter plasmids containing 5'-flanking regions of the human shelterin encoding genes (Figure 3) [9]. By performing the multiple Luc assay, the effect of β-thujaplicin on these transcription-regulatory regions were examined. The results showed that β-thujaplicin (10 μM) could induce up-regulation of relative promoter activities of the *RTEL*, *TRF1*, *TRF2*, *TERT*, *DKC1*, *TIN2*, *RAP1*(*TERF2IP*) and *TPP1*(*ACD*) genes (Figure 3). Approximate 1.5 to 2-folds increases as compared with non-treated cells were observed in a similar manner as the 396-bp of the *SIRT1* promoter (Figure 2A).

Discussion

It has been suggested that both cellular senescence and aging of organisms are accelerated by various factors, such as telomere-shortening [23-25] and DNA damaging reactive oxygen species (ROS) that are mainly generated from mitochondria [26,27]. On the other hand, an important fact for anti-aging is the demonstration that caloric restriction elongates lifespans of organisms [28], suggesting that metabolism regulatory systems could control lifespan. Genetic analyses of *C.elegans* showed that several genes encoding insulin/IGF1 receptor and transcription factor FoxO play important roles in controlling the lifespan [29]. Moreover, studies of budding yeast showed that Sir2, a member of the sirtuin proteins with an NAD^+-dependent protein

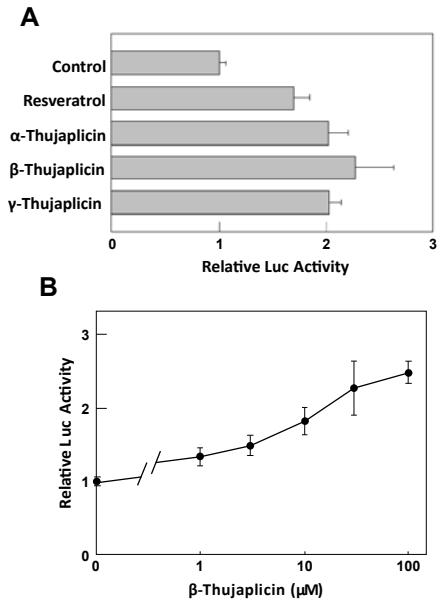

Figure 2: Effects of thujaplicins on the human *SIRT1* promoter activity. (A) The Luc reporter plasmid, pGL4-SIRT1 [6,7], was transfected into HeLa S3 cells as described under Materials and Methods. After 24 h of transfection, cells were treated with Rsv (10 μM), then harvested after a further 24 h incubation. (B) A similar (experiment was performed as in (A) with 0 to 100 μM of β-thujaplicin. The results show relative Luc activities of the indicated Luc reporter plasmid-transfected cells relative to those of non-treated cells. The values are the mean ± SD of four independent assays.

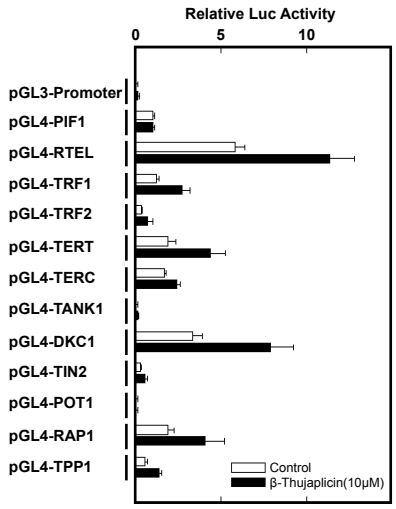

Figure 3: The effect of β-thujaplicin on the promoter activities of 5'-upstream regions of human shelterin encoding genes. Reporter plasmids (10 ng) and DEAE-dextran were spotted and dried onto each well of the 96-well culture plate. HeLa S3 cells (1 x 10^5/well) were used for transfection and incubated for further 24 h, then treated with β-thujaplicin (10 μM) for 24 h. Results show relative Luc activities from various Luc reporter transfected cells compared with that of pGL4-PIF1 transfected cells..

deacetylase activity, has silencing action on chronological aging of yeast cells [30]. Many proteins, including PGC-1α, p53, FOXO1, HIF1α, UCP2 and PPARγ, have been reported to be the targets of SIRT1, which is known as mammalian homologue of Sir2 [31]. Because these protein factors function as metabolism regulators, SIRT1 could become a key regulator of healthspan of organisms [31].

In this study, we have examined promoter activities of the 396-bp 5'-flanking region of the human *SIRT1* gene to find out its response to the treatments with three types of thujaplicins (α, β and γ) in HeLa-S3 cells. The 396-bp region has no apparent TATA-box but contains several well known transcription factor binding elements, including CREB, C/EBPβ, c-ETS, USF, SREBP1, Sp1, GATA and c-MYC binding motifs [7]. It has been shown that FOXO1, CREB, PPAR proteins and PARP2 play roles in regulation of the *SIRT1* promoter [31]. However, at present, the Rsv or β-thujaplicin-responsive elements in the *SIRT1* promoter region have not been precisely determined. Previously, it was indicated that the 5'-upstream regions of the *WRN*, *BLM*, *TERT*, *p21* (*CDKN1A*) and *HELB* genes possess one or more Sp1/GC-box elements and that they positively respond to Rsv treatment in HeLa S3 cells [6,32]. The GC-box consensus sequence of the Sp1 transcription factor binding site is: 5'-(G/T)GGGCGG(G/A)(G/A)(C/T)-3' or 5'-(G/T)(G/A)GGCG(G/T)(G/A)(G/A)(C/T)-3' [33]. It has been shown that two GC-boxes, 5'-AGGGCGGGGG-3' and 5'GGGGCGGGTC -3' (-83 to -74 and -66 to -57, respectively), play important roles in the *SIRT1* promoter activity [34]. As shown in Figure 3, the 5'-upstream regions of the *RTEL*, *TRF1*, *TRF2*, *TERT*, *DKC1*, *TIN2*, *RAP1* and *TPP1* genes positively responded to the treatment with β-thujaplicin. All of the 5'-upstream regions in the Luc-reporter vectors except pGL4-RTEL have at least one Sp1/GC-box. Although TF search analysis did not find Sp1/GC-box, 5'-CGGGCGGGAC-3', 5'-TTTCCGCCGG-3' and 5'-TGCGCGCCTC-3', namely GC-box like sequences are contained in the pGL4-RTEL. Taken together, the Sp1 binding motif is possibly one of the candidate elements that respond to β-thujaplicin. Moreover, Rsv is known to up-regulate cAMP level to activate CREB, which plays important roles in hormonal metabolism, including that of the insulin signaling system [35]. The CREB element is located in the 5'-upstream regions of the *RTEL* [21] and *TPP1* [9] genes. This suggests that the CREB element in the human *SIRT1* promoter region (-288 to -281) may respond to the β-thujaplicin treatment.

It should be noted that β–thujaplicin (hinokitiol) has been shown to stabilize transcriptional active HIF-1α in HeLa and HepG2 cells to increase transcription of the *VEGF* gene [36]. On the other hand, SIRT1 deacetylates HIF-1α to suppress its activity [37]. Therefore, the induction of *SIRT1* gene expression by β–thujaplicin might function to reduce over-stimulated HIF-1α for the maintenance of cellular homeostasis. Moreover, it has been reported that β-thujaplicin induces G1 arrest *via* down-regulation of phosphorylated Rb and Skp2 ubiquitin ligase [38]. This cell cycle arrest is accompanied with an increase of p27 and p21 protein levels. Although the precise molecular mechanisms are remain unclear, these biological properties of β-thujaplicin including transcriptional regulation and antiviral activity [16,36] might originate from its specific structure (Figure 1) that can act as a chelator of divalent metal ions [39]. In this study, we observed the up-regulation of the *SIRT1* and shelterin-encoding gene promoter activities by the treatment of β–thujaplicin. Moreover, the comparisons of 5'-upstream regions of those genes suggested that transcription factors, including Sp1, may control lifespans of organisms responding to β-thujaplicin. The core structure of the thujaplicins could be applied to design lead compounds for novel anti-aging drugs, which could simultaneously activate the *SIRT1* and shelterin-encoding gene promoter activities.

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References

1. Stefani M, Markus MA, Lin RC, Pinese M, Dawes IW, et al. (2007) The effect of resveratrol on a cell model of human aging. Ann NY Acad Sci 1114: 407-418.
2. Kaeberlein M (2010) Resveratrol and rapamycin: are they anti-aging drugs? Bioessays 32: 96-99.
3. Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, et al. (2003) Small molecule activators of sirtuins extend Saccharomyces cerevisiae life span. Nature 425: 191-196.
4. Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, et al. (2004) Sirtuin activators mimic caloric restriction and delay ageing in metazoans. Nature 430: 686-689.
5. Viswanathan M, Kim SK, Berdichevsky A, Guarente L (2005) A role for SIR-2.1 regulation of ER stress response genes in determining C. elegans life span. Dev Cell 9: 605-615.
6. Uchiumi F, Watanabe T, Hasegawa S, Hoshi T, Higami Y, et al. (2011) The effect of resveratrol on the Werner Syndrome RecQ helicase gene and telomerase activity. Curr Aging Sci 4: 1-7.
7. Uchiumi F, Tachibana H, Larsen S, Tanuma S (2012) Effect of lignin glycosides extracted from pine cones on the human *SIRT1* promoter. Pharm Anal Acta S8: 001.
8. Sfeir A, de Lange T (2012) Removal of shelterin reveals the telomere end-protection problem. Science 336: 593-597.
9. Uchiumi F, Oyama T, Ozaki K, Tanuma S (2011) Chapter 29: Characterization of 5'-flanking regions of various human telomere maintenance factor-encoding genes. In: DNA Repair (Kruman I, Ed.), InTech-Open Access Publisher, Inc, Rijeka, Croatia: 585-596.
10. Calabrese EJ, Mattson MP, Calabrase V (2010) Resveratrol commonly displays hormesis: Occurrence and biomedical significance. Hum Exp Toxicol 29: 980-1015.

11. Gogada R, Prabhu V, Amadori M, Scott R, Hashmi S, et al. (2011) Resveratrol induces p53-independent, X-linked inhibitor of apoptosis protein (XIAP)-mediated Bax protein oligomerization on mitochondria to initiate cytochrome c release and caspase activation. J Biol Chem 286: 28749-28760.

12. Lin HY, Tang HY, Davis FB, Davis PJ (2011) Resveratrol and apoptosis. Ann NY Acad Sci 1215: 79-88.

13. Nakano H, Ikenaga S, Aizu T, Kaneko T, Matsuzaki Y, et al. (2006) Human metallotionein gene expression is upregulated by β-thjaplicin: possible involvement of protein kinase C and reactive oxygen species. Biol Pharm Bull 29: 55-59.

14. Ido Y, Muto N, Inada A, Kohroki J, Mano M, et al. (1999) Induction of apoptosis by hinokitiol, a potent iron chelator, in teratocarcinoma F9 cells is mediated through the activation of caspase-3. Cell Prolif 32: 63-73.

15. Tanaka T, Satoh T, Onozawa Y, Kohroki J, Itoh N, et al. (1999) Apoptosis during iron chelator-induced differentiation in F9 embyonal carcinoma cells. Cell Biol Int 23: 541-550.

16. Byeon SE, Lee YG, Kim JC, Han JG, Lee HY, et al. (2008) Hinokitiol, a natural tropolone derivative, inhibits TNF-α production in LPS-activated macrophages via suppression of NF-κB. Planta Med 74: 828-833.

17. Manter KD, Kelsey RG, Karchesy JJ (2007) Antimicrobial activity of extractable conifer heartwood compounds toward *Phytophthora ramorum*. J Chem Ecol 33: 2133-2147.

18. Komaki N, Watanabe T, Ogasawara A, Sato N, Mikami T, et al.(2008) Antifungal mechanism of hinokitiol against *Candida albicans*. Biol Pharm Bull 31: 735-737.

19. Takahashi S, Kamiya T, Saeki K, Nezu T, Takeuchi S, et al. (2010) Structural insights into the hot spot amino acid residues of mushroom tyrosinase for the bindings of thujaplicins. Bioorg Med Chem 18: 8112-8118.

20. Zhou B, Ikejima T, Watanabe T, Iwakoshi K, Idei Y, et al. (2009) The effect of 2-deoxy-d-glucose on Werner syndrome RecQ helicase gene. FEBS Lett 583: 1331-1336.

21. Uchiumi F, Watanabe T, Tanuma S (2010) Characterization of various promoter regions of human DNA helicase-encoding genes and identification of duplicated ets (GGAA) motifs as an essential transcription regulatory element. Exp Cell Res 316: 1523-1534.

22. Uchiumi F, Hatano T, Ito H, Yoshida T, Tanuma S (2003) Transcriptional suppression of the HIV promoter by natural compounds. Antiviral Res 58: 89-98.

23. O'Sullivan RJ, Karlseder J (2010) Telomeres: protecting chromosomes against genome instability. Nat Rev Mol Cell Biol 11: 171-181.

24. Blackburn E (2006) Chapter 1: A history of telomere biology. In: Telomeres (2nd edn) (de Lang T, Lundblad V, Blackburn E, Eds.), Cold Spring Harbor Laboratory Press, NY: 1-19.

25. Vijg J (2007) Chapter 5: Genome instability and accelerated aging. In: Aging of the Genome: The Dual Role of DNA in Life and Death, Oxford University Press, NY: 151-180.

26. Robb EL, Page MM, Stuart JA (2009) Mitochondria, cellular stress resistance, somatic cell depletion and lifespan. Curr Aging Sci 2: 12-27.

27. Benz CC, Yau C (2008) Ageing, oxidative stress and cancer: paradigms in parallax. Nat Rev Cancer 8: 875-879.

28. Cavallini G, Donati A, Gori Z, Bergamini E (2008) Towards an understanding of the anti-aging mechanism of caloric restriction. Curr Aging Sci 1: 4-9.

29. Kenyon CJ (2010) The genetics of ageing. Nature 464: 504-512.

30. Kaeberlein M (2010) Lessons on longevity from budding yeast. Nature 464: 513-519.

31. Houtkooper RH, Pirinen E, Auwerx J (2012) Sirtuins as regulators of metabolism and healthspan. Nat Rev Mol Cell Biol 13: 225-238.

32. Uchiumi F, Higami Y, Tanuma S (2010) Chapter 5: Regulations of telomerase activity and *WRN* gene expression. In: Telomerase: Composition, Functions and Clinical Implications (Gagnon AN, Ed.), Nova Science Publishers, Inc, Hauppauge, NY: 95-103.

33. Wierstra I (2008) Sp1: Emerging roles-Beyond constitutive activation of TATA-less housekeeping genes. Biochem Biophys Res Commun 372: 1-13.

34. Okazaki M, Iwasaki Y, Nishiyama M, Taguchi T, Tsugita M, et al. (2010) PPARβ/δ regulates the human SIRT1 gene transcription via Sp1. Endocr J 57: 403-413.

35. Altarejos JY, Montminy M (2011) CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. Nat Rev Mol Cell Biol 12: 141-151.

36. Lee MJ, Kim JW, Yang EG (2010) Hinokitiol activates hypoxia-inducible factor (HIF) pathway through inhibition of HIF hydroxylases. Biochem Biophys Res Commun 396: 370-375.

37. Lim JH, Lee YM, Chun YS, Chen J, Kim JE, et al. (2010) Sirtuin 1 modulates cellular responses to hypoxia by deacetylating hypoxia-inducible factor 1α. Mol Cell 38: 864-878.

38. Liu S, Yamauchi H (2009) p27-Associated G1 arrest induced by hinokitiol in human malignant melanoma cells is mediated via down-regulation of pRb, Skp2 ubiquitin ligase, and impairment of Cdk2 function. Cancer Lett 286: 240-249.

39. Krenn BM, Gaudernak E, Holzer B, Lanke K, Van Kuppeveld FJM, et al. (2009) Antiviral activity of the zinc ionophores pyrithione and hinokitiol against picornavirus infections. J Virol 83: 58-64.