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

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Abstract

Resveratrol (Rsv), which 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 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 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).
penicillin-streptomycin at 37°C in a humidified atmosphere with 5% CO₂.

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-phosphate (pH 7.8), 2 mM DTG, 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.

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.

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, POT1, 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 1: The structures of trans-Resveratrol and thujaplicins.](image_url)

![Figure 2: Effects of thujaplicins on the human SIRT1 promoter activity.](image_url)

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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 10⁶/well) were used for transfection and incubated for further 24 h, then treated with β-thujaplicin (10 μM) for 24 h. Results show relative Lu activities from various Lu 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 PPARY, 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, PPARY 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(G/T)GGGCCC(A/G)/(A)/(G)/(A)/(C)/(T)-3′ or 5′-G(G/T)/(G/A)GGGCCC(G/C)/(G/A)/(G)/(A)/(C)/(T)-3′ [33]. It has been shown that two GC-boxes, 5′-AGGGGCGGG-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, TRFI, TRF2, TERT, DKC1, TIN2, RAPI and TPP1 genes positively responded to the treatment with β-thujaplicin. Among the 5′-upstream regions of 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′-CGGGCCGGGAC-3′, 5′-TTTCCGCCGGG-3′ and 5′-GGGGCGGGTC-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
