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Enantioselective induction of *SIRT1* gene by syringaresinol from *Panax ginseng* berry and *Acanthopanax senticosus* Harms stem

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ABSTRACT

Syringaresinol exists either exclusively as one enantiomer or enantiomeric mixtures in plant foods. We found that (+)-syringaresinol, but not (−)-syringaresinol, upregulates *silent information regulator two ortholog 1* (*SIRT1*) gene expression, and thus, *Panax ginseng* berry with predominantly high contents of (+)-syringaresinol exhibits higher activity in inducing *SIRT1* gene expression than *Acanthopanax senticosus* Harms stem with almost equal proportion of the two enantiomers. These findings highlight the importance of the absolute configuration of syringaresinol for the biological activity.

Lignans are phytoestrogens occurring in various plant foods.[1] They exhibit various health-promoting effects such as antioxidant,[2] antitumorigenic,[3] and antidiabetic effects.[4] Syringaresinol (4,4′-(1*S*,3a*R*,4*S*,6a*R*)-tetrahydro-1*H*,3*H*-furo[3,4-*c*]furan-1,4-diyl-bis(2,6-dimethoxyphenol) is a lignan with several pharmacological functions, including an inhibitory effect on cyclic AMP phosphodiesterase,[5] a prolonging effect on exercise time[6] and a promoting effect on vascular relaxation.[7] Syringaresinol exists as a single pair of enantiomers with *S*-(+) (**1**) and *R*-(−) (**2**) configurations (Fig. 1).[8] (−)-Syringaresinol (**2**) is the dominating enantiomer in sesame seeds and camelina seeds, whereas (+)-syringaresinol (**1**) is present in relatively high proportions in various berry seeds.[8] Chiral enantiomers have similar physicochemical properties but reveal different activity and toxicity, as cellular receptors and enzymes usually favor one enantiomer over the other.[9,10] Considering the possibility for enantiomers to elicit totally different biological effects, it is important to know the enantiomeric composition of syringaresinol in different plant foods. However, the enantiomeric composition of syringaresinol and its enantioselective activities in plant foods are not well understood. Our previous study showed that syringaresinol, isolated from *Panax ginseng* berry pulp, delayed endothelial cellular senescence via upregulation of *SIRT1* gene expression.[11] SIRT1 is a nicotinamide (NAD+)-dependent deacetylase that plays a critical role in age-related metabolic changes, including lipid metabolism, insulin secretion, neuroprotection, and cardioprotection.[12] These aspects of SIRT1 function have raised a broad interest in SIRT1-targeted pharmaceutical interventions and functional foods, particularly against age-associated disorders. Various small-molecule activators of SIRT1, including the plant-derived polyphenolic compound resveratrol, have been used to improve

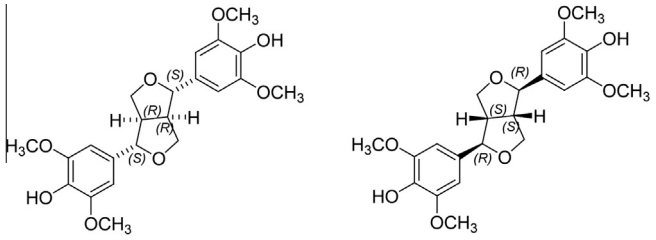

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Figure 1. Chemical structures of syringaresinol enantiomers.

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metabolic complications.[13] However, little is presently known about upregulation of *SIRT1* gene expression by dietary compounds. In this study, we investigated the enantiomeric ratio of syringaresinol samples isolated from *Panax ginseng* berry pulp (GBP) and *Acanthopanax senticosus* Harms stem (SHS), known as high producer of syringaresinol as compared with other plants,[14] and the biological activities of **1** and **2** to evaluate its enantioselective activities.

Freshly harvested 4-year-old Korean GBP was sequentially extracted with 70% ethanol, followed by solvent partitioning with water-saturated butanol. SHS was extracted with 70% methanol at room temperature, followed by partitioning with ethyl acetate. Each the ethyl acetate-soluble extract of SHS (50 g) and the butanol-soluble fraction of GBP (194 g) was fractionated on ODS reversed-phase column chromatography using MeOH–water (10:90 to 95:5, v/v). Each eluate containing syringaresinol was chromatographed on a Sephadex LH-20 column with 50% aqueous methanol and then was purified with preparative silica gel thin layer chromatography developed with $CHCl_3$–MeOH (10:1, v/v), followed by preparative HPLC with 33% aqueous methanol to give syringaresinol (17 mg for GBP and 300 mg for SHS) (the flow chart of isolation is depicted in Supporting information).

The structure of syringaresinol isolated from GBP and SHS was confirmed via a comparison of its mass and NMR spectral data with those of authentic reference compound. Our previous experiments revealed that GBP syringaresinol induced *SIRT1* mRNA levels up to two-fold, as compared to untreated controls. Furthermore, we found that a syringaresinol-responsive element is present in the region between −377 and −533 base pair (bp) from the *SIRT1* transcription initiation site.[11] Next, we investigated the effects of SHS syringaresinol on *SIRT1* gene expression. Human umbilical vein endothelial cells (HUVECs) treated with SHS syringaresinol showed lower levels of *SIRT1* expression as compared to cells treated with GBP syringaresinol (Fig. 2), suggesting that GBP syringaresinol was more active than SHS syringaresinol in induction of *SIRT1* expression. Given that the difference in enantiomeric ratio affects biological activities of lignans,[8,10,15] we speculated that the different enantiomeric composition of syringaresinol isolated from GBP and SHS might contribute to differential effects of these two syringaresinol samples on *SIRT1* expression. To test this, enantiomeric separation of the syringaresinol obtained from GBP and SHS was performed using a supercritical fluid chromatography (SFC) system with a Chiralpak IB column. As shown in Figure 3A, reference compound **1** is the first peak on the graph, and **2** is the second peak. GBP contained predominantly **1**, whereas SHS contained **1** and **2** in almost equal proportions with **1** slightly dominating (Fig. 3B and C). Physical parameters of the enantiomers from ginseng berry were determined to be **1** ($[\alpha]_D$ +40.9°, *c* 0.1, $CHCl_3$) and **2** ($[\alpha]_D$ −38.5°, *c* 0.1, $CHCl_3$), occurring at a ratio of 97:3 according to the chiral chromatography elution profile.

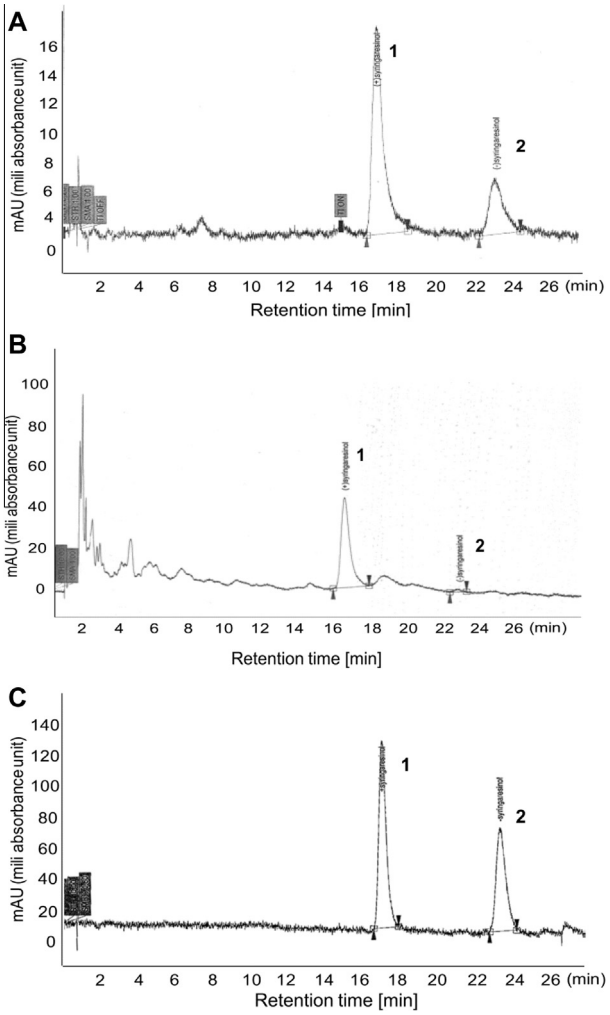

Figure 3. (A) Chiral SFC analysis of **1** and **2** reference compounds. (B) Chiral SFC analysis of GBP syringaresinol sample. (C) Chiral SFC analysis of the SHS syringaresinol sample.

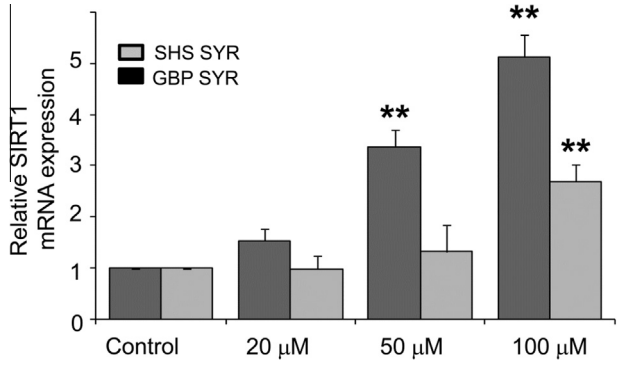

Figure 2. Effects of GBP syringaresinol and SHS syringaresinol on *SIRT1* expression. *SIRT1* mRNA levels were measured in HUVECs cultured with various doses of either GBP or SHS syringaresinol by real-time reverse transcriptase PCR. *SIRT1* mRNA levels were quantified and normalized to *GAPDH* mRNA levels. All results are presented as mean ± SD from six independent experiments. $^{**}P < 0.001$ versus the control. SYR: syringaresinol.

Next, we examined the enantioselective effects of syringaresinol on *SIRT1* gene expression using synthesized **1** or **2**. Synthetic **1** treatment significantly increased *SIRT1* mRNA levels in a concentration-dependent manner (Fig. 4A). In contrast, *SIRT1* mRNA levels were not altered in HUVECs treated with **2**. To further confirm the enantioselective activation of the *SIRT1* gene by syringaresinol, HUVECs were transiently transfected with a luciferase reporter construct driven by the promoter (nucleotides −533 to −1) of the *SIRT1* gene, and luciferase activity was measured following treatment with either **1** or **2**. Synthetic **1** treatment induced luciferase activity in a dose-dependent manner, whereas **2** failed to cause an obvious change in luciferase activity compared to the untreated cells (Fig. 4B). No obvious enantioselective cytotoxicity of syringaresinol in HUVECs was observed (see Fig. 1 in Supporting information). Hence, these results indicate that syringaresinol led to the induction of *SIRT1* expression in an enantiospecific manner. To identify a mechanism underlying enantioselective activation of SIRT1 by syringaresinol, further studies have been performed. Given that the stimulatory effects of syringaresinol on

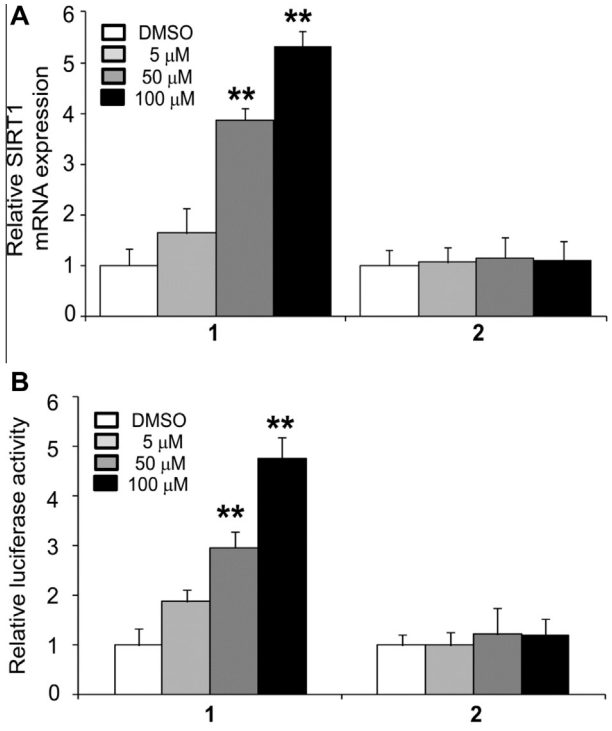

Figure 4. Enantioselective effects of syringaresinol on *SIRT1* expression and promoter. (A) *SIRT1* mRNA levels in HUVECs treated with different doses of either **1** or **2**. *SIRT1* mRNA levels were quantified and normalized to *GAPDH* mRNA levels. (B) HUVECs were transfected with a *SIRT1* promoter construct. At 24 h after transfection, cells were treated with DMSO, **1** or **2** (5, 50, and 100 μM) for 24 h, and luciferase activities were measured. The relative luciferase activity is the ratio of luciferase/total protein levels normalized to DMSO-treated cells. All results are presented as mean ± SD from six independent experiments. $^{**}P < 0.001$ versus the control (DMSO).

SIRT1 expression depended on FOXO3a,[11] we investigated the enantioselective effect of syringaresinol on recruitment of FOXO3a to the *SIRT1* promoter. Our experiment showed that (+)-syringaresinol, but not (−)-syringaresinol, induced binding of FOXO3a to the *SIRT1* promoter (see Fig. 2 in Supporting information). Furthermore, surface plasmon resonance (SPR) analysis showed that (+)-syringaresinol, but not (−)-syringaresinol, directly interacted with FOXO3 in vitro (see Fig. 3 in Supporting information). Although the exact molecular mechanism responsible for the enantiospecific effect of syringaresinol on *SIRT1* expression was not determined, our preliminary data showed that syringaresinol might exert its enantioselective effects on *SIRT1* gene expression though an enantioselective interaction with FOXO3 and its recruitment to the *SIRT1* promoter.

In conclusion, GBP syringaresinol had greater potential to induce *SIRT1* gene expression than SHS syringaresinol, at least in part due to a high content of **1**:**1** is responsible for the *SIRT1* activator of syringaresinol, while **2** is ineffective. Although the underlying mechanisms remain elusive, the findings of this study highlight the importance of the absolute configuration at stereocenter of the selected syringaresinol for controlling the biological activity.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.11.045.

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