IDENTIFICATION OF WATER-SOLUBLE EXTRACT ISOLATED FROM A MEDICINAL PLANT, *ANGELICA GIGAS* AS SELECTIVE ANTAGONIST AGAINST 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN USING TRANSCRIPTIONAL REGULATION ANALYSIS SYSTEM

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Introduction

The arylhydrocarbon receptor (AhR) mediated signaling pathway is the target of potent environmental contaminants, which results in cytotoxicity and genotoxicity of an organism. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), one of the most toxic man-made compounds, is widely dispersed in the environment and bioaccumulates of animal and human [1-3].

Although the toxicity of dioxin has been widely reported, the inhibition studies focused on dioxin-XRE mediated gene activation at the transcriptional level have been poorly reported. Here, we report the antagonistic effects of medicinal plant extracts prepared from *Angelica gigas* on TCDD-XRE mediated activation of gene expression by using our newly developed transcriptional regulation analysis system (TRAS) at the cellular level. For this, on the basis of action mechanism of TCDD-XRE interaction, we established the transformed cell line TRAS-XRE HepG2, which is highly sensitive to TCDD treatment. Then, using our natural product library including medicinal plants, herbs, natural tonics and so on, we carried out antagonist screening against TCDD-mediated action. As a result, we could identify *Angelica gigas* extracts (AE) as one of the best fungible candidates for antagonist against TCDD-XRE mediated downstream signaling pathway.

Methods and Materials

All plasmids were constructed according to the standard DNA cloning procedures and PCR methods [4]. For the construction of TRAS-enhancer vector, *Bgl*II-*Xba*I fragment (including ORF of *luciferase* gene) of pGL3-basic vector (Promega, Madison, WI) was inserted into *Bgl*II-*Xba*I site

within pcDNA3 vector (Invitrogen, Carlsbad, CA). Subsequently, the multiple cloning sites (MCS) were inserted into *Bgl*II - *Hind*III site of cloned pGL3-basic fragment within pcDNA3. The TRAS-XRE was constructed as follow; enhancer region of hCMV immediate early promoter was eliminated, then the rest minimal part of hCMV immediate early promoter sequence was synthesized with additional 5'-*Bam*HI and *Hind*III-3' linker and was cloned into *Bam*HI – *Hind*III site of TRAS-Enhancer plasmid. The plasmid TRAS-XRE was generated using fifteen copies of CYP1A1 derived XRE. In order to establish the stable transformants expressed in HepG2 cells, we transfected the recombinant plasmids TRAS-XRE, TRAS-p53 and TRAS-NFkB into HepG2 cells, respectively, according to the lipofectin method [4]. The neomycin-resistant colonies for TRAS-XRE, TRAS-p53 and TRAS-NFκB were isolated and proliferated, respectively, for the experimental use. The confirmation of transformed cell lines, HepG2-TRAS-XRE, HepG2-TRAS-p53 and HepG2-TRAS-NFκB was carried out by polymerase chain reaction (PCR).

Results and Discussion

The potent toxicity of dioxin has been extensively studied concerning the mechanism of *CYP1A1* induction by TCDD and its involvement in the metabolism of xenobiotics. However, little is known about the antagonizing agents against dioxin on the basis of its mechanism of action. Our studies are focused on the selection of effective candidate act as antagonist on the TCDD-XRE interaction at the transcriptional level.

In order to select the active fraction(s) that inhibit the TCDD-XRE mediated gene activation, we isolated extracts from the natural product library which was consisted of various plant species including medicinal plants, herbs, natural tonics and so on. These water-soluble extracts were subjected to our screening system, HepG2-TRAS-XRE, which stably expresses the *luciferase* gene in cells. To develop the screening system TRAS, we generated the recombinant plasmid TRAS-Enhancer using pGL3-basic and pcDNA3 by inserting multiple cloning sites and minimal part of hCMV immediate early promoter as described in materials and methods (Fig. 1). Selection procedure was carried out for a month, and then the transformed cell lines were confirmed by PCR using MCS specific primer (5`-primer) and cDNA specific primer of the *luciferase* gene (3`-primer) (Fig. 2A). Fig. 2B clearly shows that the PCR products were appeared at approximately 703-, and 831-bp location and thus specific induction was observed in every construct. These transformed cell lines were maintained in 96-well culture plate, and the TRAS-XRE HepG2 cells were used in TCDD studies. Fig. 3 shows induction profile of luciferase activities by TCDD treatment. TCDD was added to culture medium with a concentration range of 10^{-4} nM to 10^{2} nM, and cells were maintained for 12h and the luciferase activity of each TCDD-treated cells were determined. The peak of induction appeared around 10^{-1} nM concentration of TCDD and showed the steady-state level above 1 nM concentration (Fig. 3). This induction pattern was similar to the

induction profile of previous reports. But the concentration reached at maximal induction was much lower than that of other observations. This phenomenon of sensitivity difference could be explained by multimerized *cis*-elements for XRE and the transformed cell line TRAS-XRE HepG2 system. Then, we tested which water-soluble extracts had an antagonistic function on the TCDD-mediated induction. 100 ug/ml of each extracts were treated with or without TCDD on TRAS-XRE HepG2 cells. Likewise, as a representative of cellular marker, TRAS-p53 HepG2 and TRAS-NFkB HepG2 were treated with 100 ug/ml of extracts to examine the cellular effects of library samples. After 24h, the luciferase activity of each cells were determined. The AE inhibited luciferase activity to the control level relative to only TCDD-treated cells (Fig. 4A). In contrast to the marked inhibition in TRAS-XRE HepG2 cells, this treatment resulted in no alteration of luciferase activity in TRAS-p53 HepG2 and TRAS-NFkB HepG2 cells (Fig. 4B). These results imply that the AE itself did not induce significant changes in cellular level. To assess the potentiality of antagonistic effect of AE in TCDD activity, a similar experiment was performed. Fig. 5 demonstrates that the AE inhibited TCDD-mediated activation up to 10 nM of TCDD concentration. This result confirmed again the AE-specific repression on the TCDD-XRE mediated gene activation.

Our TRAS, which is developed to measure the dioxin contamination in food and other contaminants with higher sensitivity, may serve as a useful screening and detection system for dioxin-related areas. Many studies remain to be done concerning the antagonistic function of AE to dioxin-XRE mediated signaling, such as identification of specific compounds, the working stage and mode of action between each other.

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References

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Figure legend

Fig. 1. Map of TRAS-Enhancer vector for construction of TRAS. *Luciferase* gene of pGL3-basic vector was inserted into *Bgl*II-*Xba*I site of pcDNA3 vector. Then inserted the synthesized hCMV and MCS sequences. Appropriate DNA fragments can be inserted in MCS and be used in TRAS.

Fig. 2. Confirmation of transformed TRAS-cell line. A. Schematic diagram of stably-inserted TRAS-vectors. Predicted products size and location of primers for PCR are shown. B. Whole genomic DNAs of stable cell lines were prepared and subjected into PCR. Genomic DNAs from stable cell lines showed the bands as predicted (Lane 3-5).

Fig. 3. Dose-dependent effect of TCDD on HepG2-TRAS-XRE. TCDD activated TRAS-XRE from 10^{-4} nM and showed the greatest effect at 0.1 nM (about 10 fold).

Fig. 4. Effect of Angelica gigas extract (AE) on the activity of TCDD on TRAS-XRE. A. AE was prepared and treated on HepG2-TRAS-XRE. 0.1 nM of TCDD and 100 ug/ml of AE were used as indicated. B. Effect of AE on TRAS-p53 and TRAS-NFκB. 100 ug/ml of AE was used.

Fig. 5. Antagonistic effect of AE on TCDD. Fixing the concentration of AE, the concentration of TCDD on HepG2-TRAS-XRE was changed. AE successfully repressed the activity of TCDD up to 10 nM.

Figure 1.

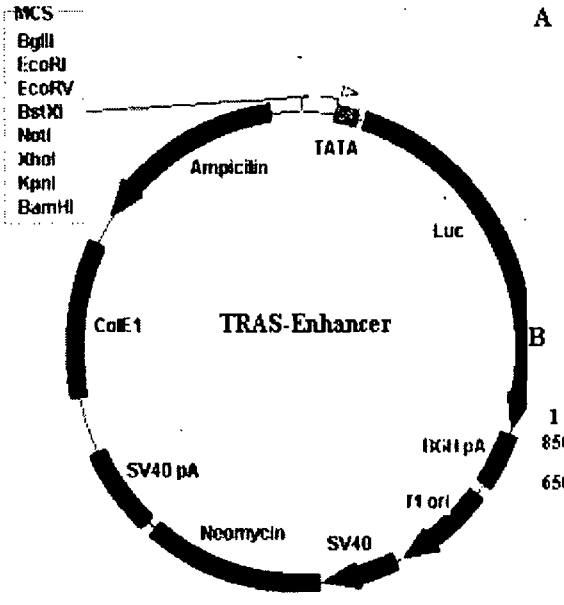

Figure 2

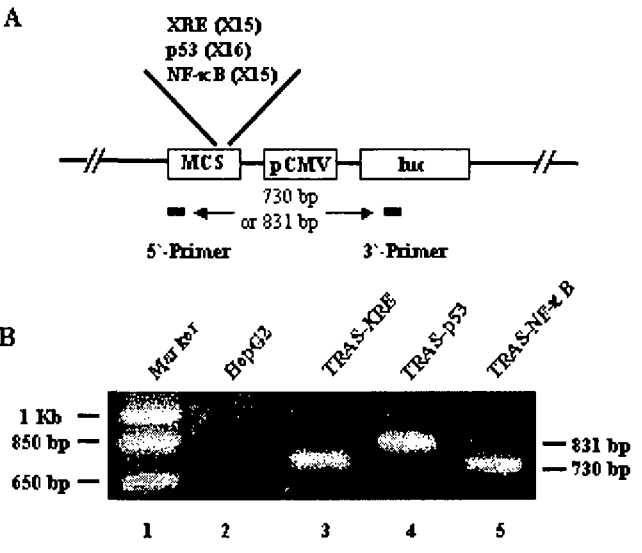

Figure 3

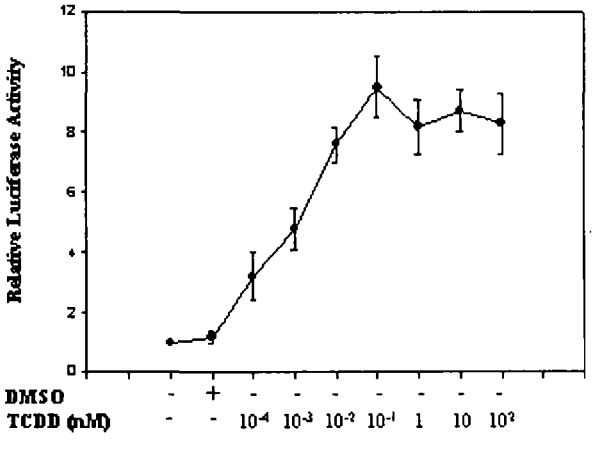

Figure 4

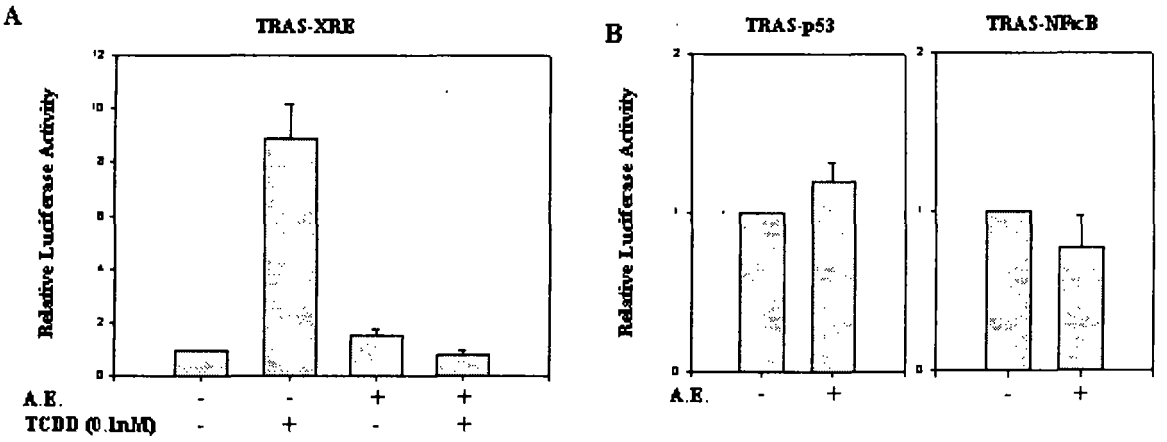

Figure 5