Preferential Blockade of Dioxin-Induced Activation of the Aryl Hydrocarbon Receptor by *Antrodia camphorata*

Mai MUKAI,^a Kunihiro HAYAKAWA,^a Maro OKAMURA,^a Yasuhiro TAGAWA,^a Shotaro NAKAJIMA,^a Yukinori SAITO,^a Shuhei TAKAHASHI,^a Jian YAO,^a Daisuke NISHIMURA,^{b,c} Masahito SUGI,^{b,c} Masaji MATSUNAGA,^{b,c} and Masanori KITAMURA^{*a}

^aDepartment of Molecular Signaling, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi; Chuo, Yamanashi 409–3898, Japan; ^bHokkaido Research Institute, Nissei Bio Co., Ltd.; Eniwa, Hokkaido 061–1374, Japan; and ^cGene Trophology Research Institute; Eniwa, Hokkaido 061–1374, Japan.

Received April 10, 2009; accepted June 10, 2009; published online June 15, 2009

Halogenated and polycyclic aromatic hydrocarbons are widely distributed pollutants in environments. These toxic substances activate the aryl hydrocarbon receptor (AhR) and thereby cause a broad spectrum of pathological changes. Development of AhR inhibitors will be useful for prevention of diseases caused by AhR activation. Using the dioxin responsive element (DRE)-based sensing *via* secreted alkaline phosphatase (DRESSA), we examined effects of *Antrodia camphorata*, a mycelial extract, on the activation of AhR by halogenated and polycyclic aromatic hydrocarbons. We found that *Antrodia camphorata* markedly suppressed activation of AhR triggered by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). In contrast, activation of AhR by polycyclic aromatic hydrocarbons (benzo[*a*]pyrene and 3-methylcholanthrene) was inhibited only modestly by this mycelium. Similarly, *Antrodia camphorata* only mildly attenuated activation of AhR by cigarette smoke that contains polycyclic aromatic hydrocarbons. Consistent with these results, Northern blot analysis revealed that DRE-driven exogenous and endogenous gene expression triggered by TCDD was abolished by *Antrodia camphorata*, whereas it did not substantially affect DRE-induced transcription triggered by benzo[*a*]pyrene, 3-methylcholanthrene or cigarette smoke. We also found that the inhibitory effect of *Antrodia camphorata* on TCDD-induced AhR activation was ascribed to neither down-regulation of AhR, down-regulation of the AhR nuclear translocator, nor up-regulation of the AhR repressor. These results suggest that *Antrodia camphorata* preferentially inhibits AhR activation and DRE-dependent gene expression triggered by dioxin.

Key words aryl hydrocarbon receptor; *Antrodia camphorata*; dioxin; polycyclic aromatic hydrocarbon; cigarette smoke

Dioxin and polycyclic aromatic hydrocarbon (PAH) are widely distributed pollutants in environments. These toxic substances activate the aryl hydrocarbon receptor (AhR) and thereby cause a broad spectrum of pathologies in mammals, including carcinogenesis, immunotoxicity, endocrine dysregulation and reproductive/developmental abnormalities.^{1,2)} Cigarette smoke contains various PAH such as benzo[*a*]pyrene (B[*a*]P) and has high levels of dioxin-like potential that triggers the AhR pathway *in vitro* and *in vivo*.^{3,4)} It may be responsible for the development of cigarette smoke-related pathologies, including carcinogenesis, inflammation, atherosclerosis, immune dysfunction and teratogenesis.⁵⁾

When cells are exposed to dioxin and PAH, these AhR ligands pass through the cell membrane and bind to AhR in the cytoplasm. The ligand–AhR complex translocates into the nucleus and is heterodimerized with the AhR nuclear translocator (ARNT). These AhR–ARNT complexes bind to a particular DNA motif, the dioxin responsive element (DRE), leading to transcriptional induction of downstream genes including cytochrome P450 1A1 (*CYP1A1*).⁶⁾ The AhR repressor (AhRR) is also one of the target genes for AhR–ARNT. AhR and AhRR, therefore, constitute a negative feedback loop of the dioxin pathway, where the AhRR induced by AhR/ARNT, in turn, inhibits the function of AhR.⁷⁾ Using AhR-null mutant mice, previous reports showed a critical role of AhR in the toxicity of dioxin and PAH.^{8–10)} On the other hand, constitutive activation of AhR *via* genetic manipulation caused tumorigenesis and immune abnormality in mice even without exposure to xenobiotic lig-

ands.^{11–14)} Based on this current knowledge, development of AhR antagonists may be useful for prevention and treatment of diseases associated with aberrant activation of AhR.⁵⁾

Previous studies showed that mycelia contain bioactive substances that modulate various cell functions. For example, certain mycelial extracts have stimulatory actions on immune responsiveness; *e.g.*, activation of mononuclear cells and expression of cytokines and their cognate receptors.¹⁵⁾ Among those, *Antrodia camphorata* is a medicinal mushroom that has a broad range of pharmacological potential including anticancer, immunomodulatory and anti-inflammatory effects.^{16–18)} However, currently, there are no reports showing the potential of *Antrodia camphorata* on the regulation of the AhR pathway. In the present investigation, we examined an effect of *Antrodia camphorata* on the activation of AhR triggered by dioxin and PAH. For this purpose, we used the DRE-based sensing *via* secreted alkaline phosphatase (DRESSA) bioassay.¹⁹⁾ It is a fast and sensitive assay that can detect and quantify activation of AhR by dioxins and dioxin-like chemicals including PAH.^{19,20)} In this approach, a murine hepatoma cell line Hepa-1c1c7 was stably transfected with a secreted alkaline phosphatase (*SEAP*) gene under the control of the DRE consensus sequences. The established sensor cells secrete SEAP following exposure to AhR agonists in a dose-dependent manner.^{19,20)}

Using this approach, we here demonstrate that *Antrodia camphorata* inhibits activation of AhR and induction of DRE-dependent gene expression by dioxin and that this inhibition is independent of down-regulation of AhR, down-regulation of the ARNT or up-regulation of the AhRR. We also

* To whom correspondence should be addressed. e-mail: masanori@yamanashi.ac.jp

show that the inhibitory effect of *Antrodia camphorata* is relatively specific on dioxin and that AhR activation triggered by PAH and cigarette smoke is only minimally affected by this mycelium.

MATERIALS AND METHODS

Reagents A mycelial sample was prepared from *Antrodia camphorata* by Nissei Bio Co., Ltd. (Eniwa, Hokkaido, Japan). In brief, freeze-dried mycelia of *Antrodia camphorata* were dissolved in phosphate-buffered saline (PBS) at 10 mg/ml and boiled at 65 °C for 30 min. This material was further diluted by culture medium to 10–100 µg/ml and used for experiments. B[a]P and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and 3-methylcholanthrene (3MC) was obtained from Sigma-Aldrich Japan (Tokyo, Japan).

Preparation of Cigarette Smoke Extract (CSE) To prepare CSE, mainstream smoke was made from 10 mg-tar cigarettes using constant vacuum flow (10.5 l/min) and collected in a glass bottle, as described previously.²¹⁾ The smoke from 20 cigarettes was dissolved in 500 ml PBS with vigorous shaking. The prepared CSE was stored at –80 °C until use (designated as 100% CSE). The AhR-activating potential of the prepared CSE was comparable to that of 1.6 ng/ml TCDD.

Cells The murine hepatoma cell line Hepa-1c1c7 was purchased from American Type Culture Collection (Manassas, VA, U.S.A.). The reporter clone HeXS34 was established by transfection of Hepa-1c1c7 cells with pXRE-SEAP that introduces a SEAP gene under the control of 2 copies of the xenobiotic responsive element (XRE) consensus sequence, as described previously.²²⁾ HeSS8 cells were also established by transfection of Hepa-1c1c7 cells with pSV40-SEAP (BD Biosciences, Palo Alto, CA, U.S.A.) that introduces a SEAP gene under the control of the simian virus 40 promoter.²²⁾ Cells were maintained in α -minimum essential medium (α -MEM; Invitrogen, Carlsbad, CA, U.S.A.) supplemented with 5% fetal bovine serum (FBS).

Pharmacological Treatment Cells were seeded in 96-well plates (10000 cells/well), pretreated with the extract of *Antrodia camphorata* at 10–100 µg/ml for 30 min and then treated with ED₅₀ concentrations for TCDD (25 pM),³⁾ B[a]P (15 nM),²⁰⁾ 3MC (5 nM)²⁰⁾ or CSE (1%)³⁾ in the presence of *Antrodia camphorata* for 24 h. After the treatments, culture media and cells were subjected to; i) chemiluminescent assay to evaluate SEAP activity, and ii) formazan assay to assess the number of viable cells, respectively. α -MEM containing 1% FBS was generally used for experiments.

SEAP Assay Activity of SEAP in culture media was evaluated by a chemiluminescent method using Great EscAPE SEAP Detection Kit (BD Biosciences).¹⁹⁾ In brief, 5 µl of culture medium was mixed with 15 µl of 1× dilution buffer and incubated for 30 min at 65 °C. After the incubation, samples were mixed with 20 µl of assay buffer containing L-homoarginine, left at room temperature for 5 min and then added with 20 µl of chemiluminescent enhancer containing 1.25 mM CSPD as a substrate. After incubation in dark for 30 min, the samples were subjected to analysis using a luminometer (Gene Light 55; Microtech Niton, Chiba,

Japan).

Formazan Assay The number of viable cells was assessed by a formazan assay using Cell Counting Kit-8 (Dojindo Laboratory, Kumamoto, Japan), as described previously.³⁾ In brief, after collecting culture media for chemiluminescent assays, cells in 96-well plates were incubated at 37 °C for 1.5 h in medium containing 10% Cell Counting Kit-8 assay solution. Absorbance (450 nm) of formazan generated from water-soluble tetrazolium (WST-8) was measured by Spectra Max 340 (Nihon Molecular Devices, Tokyo, Japan).

Northern Blot Analysis Total RNA was extracted by a single-step method, and Northern blot analysis was performed as described before.²³⁾ As probes, cDNAs for SEAP (BD Biosciences), AhR,²⁴⁾ ARNT,⁷⁾ AhRR,⁷⁾ and CYP1A1²⁵⁾ were radio-labeled and used for hybridization. Expression of glyceraldehyde-3-phosphate dehydrogenase was used as a loading control.

Statistical Analysis Data were expressed as means $\pm$ S.E., and statistical analysis was performed using the non-parametric Mann-Whitney *U* test to compare data in different groups. *p* value <0.05 was considered to indicate a statistically significant difference. Assays were performed in quadruplicate.

RESULTS

Inhibition of AhR Activation by *Antrodia camphorata*

Using the DRESSA bioassay, an effect of *Antrodia camphorata* on dioxin-induced activation of AhR was examined. The HeXS34 reporter cells were exposed to TCDD in the absence or presence of *Antrodia camphorata*. After the treatment, culture media and cells were subjected to chemiluminescent assay and formazan assay to evaluate SEAP activity and cell viability, respectively. As shown in Fig. 1A, treatment with *Antrodia camphorata* inhibited TCDD-induced activation of AhR in a dose-dependent manner. Significant suppression was observed at concentrations ≥ 10 µg/ml. At 100 µg/ml, *Antrodia camphorata* suppressed the activation of AhR to $14.6 \pm 0.9\%$ (vs. 100% in *Antrodia camphorata*-untreated cells; 85.4% suppression). To exclude a possibility that *Antrodia camphorata* directly affects activity of SEAP, recombinant SEAP was mixed with *Antrodia camphorata* and incubated for 24 h. The result showed that *Antrodia camphorata* did not affect activity of SEAP (Fig. 1B). Some AhR antagonists have weak agonistic potential,²⁶⁾ and *Antrodia camphorata* also caused weak but significant activation of AhR (1.9 fold at 100 µg/ml; Figs. 1C–E).

AhR is activated not only by dioxin but also by various PAH. Using the same reporter system described above, we next examined whether *Antrodia camphorata* inhibits activation of AhR by B[a]P and 3MC. As shown in Fig. 1C, B[a]P markedly induced activation of AhR, and *Antrodia camphorata* mildly increased AhR activity at 10–25 µg/ml. Only at the highest concentration (100 µg/ml), activation of AhR was significantly suppressed. The extent of suppression was modest and only 18.7%. Consistent with this result, 3MC induced activation of AhR, and *Antrodia camphorata* modestly increased AhR activity at 10–25 µg/ml. Only at 100 µg/ml, activation of AhR was significantly depressed by *Antrodia camphorata* (Fig. 1D). Like in B[a]P-induced AhR activa-

Fig. 1. Inhibition of AhR Activation by *Antrodia camphorata*

(A) HeXS34 cells in 96-well plates were exposed to 25 pM TCDD in the absence or presence of 10–100 µg/ml *Antrodia camphorata* for 24 h. After the treatment, culture media were subjected to chemiluminescent assay to evaluate SEAP activity. At the end of incubation, the number of viable cells was evaluated by the formazan assay, and activity of SEAP was normalized by the number of viable cells (SEAP/WST). Relative percentages vs. the value of the cells treated with TCDD alone are shown. (B) Recombinant SEAP was mixed with *Antrodia camphorata* at 100 µg/ml, incubated at 37°C for 24 h and subjected to chemiluminescent assay. (C–E) The cells were exposed to 15 nM B[a]P (C), 5 nM 3MC (D), 1% CSE (E) or 10 nM TCDD (F) in the absence or presence of different concentrations of *Antrodia camphorata* for 24 h. After the treatment, culture media and cells were subjected to chemiluminescent assay and formazan assay, respectively. Asterisks indicate statistically significant differences ($p < 0.05$). NS, not statistically significant. RLU, relative light unit.

tion, the extent of suppression was only modest, approximately 26.3%. These results suggest that *Antrodia camphorata* preferentially inhibits activation of AhR by TCDD, but not PAH.

Cigarette smoke contains various PAH such as B[a]P and has the high level of potential that triggers the AhR pathway. We further tested whether *Antrodia camphorata* suppresses activation of AhR by cigarette smoke. As shown in Fig. 1E, CSE markedly induced activation of AhR, and higher

dosages of *Antrodia camphorata* (50–100 µg/ml) significantly attenuated the activation of AhR. Like B[a]P and 3MC, however, the extent of suppression was only modest; approximately 21.2% at 100 µg/ml.

Antrodia camphorata inhibited TCDD-induced AhR activation more efficiently than that by PAH. If *Antrodia camphorata* functions as an antagonist of AhR, it may be simply ascribed to different concentrations of TCDD (25 pM) and PAH (5–15 nM). To examine this possibility, an effect of

Antrodia camphorata on the activation of AhR was tested in the cells exposed to a high concentration of TCDD (10 nM). The result showed that, in contrast to the strong inhibition shown in Fig. 1A, the suppressive effect of *Antrodia camphorata* was much weaker when the cells were exposed to the high concentration of TCDD (Fig. 1F).

Lack of Induction of ER (Endoplasmic Reticulum) Stress by *Antrodia camphorata* In SEAP-based reporter assays, activity of extracellular SEAP may be down-regulated by ER stress.²⁷⁾ The decrease in SEAP activity by ER stress is caused by abnormal post-translational modification, accelerated degradation and reduced secretion of SEAP protein. To confirm that the reduction in SEAP by *Antrodia camphorata* was not due to induction of ER stress, HeSS8 cells that express SEAP under the control of a constitutively active viral promoter were used. HeSS8 cells were stimulated with TCDD in the presence of *Antrodia camphorata*, and the culture media and cells were subjected to SEAP assay and formazan assay, respectively. As shown in Fig. 2, *Antrodia camphorata* did not significantly attenuate SEAP activity even at the highest concentration. This result confirmed that the down-regulation of SEAP by *Antrodia camphorata* was not due to induction of ER stress or other nonspecific effects.

Effects of *Antrodia camphorata* on AhR-Dependent Gene Expression To further confirm our conclusion, we investigated effects of *Antrodia camphorata* on AhR-dependent gene expression triggered by dioxin, PAH and cigarette smoke. HeXS34 cells were exposed to TCDD, B[a]P, 3MC or CSE in the absence or presence of *Antrodia camphorata*,

and expression of SEAP mRNA was evaluated. Northern blot analysis revealed that all of the AhR agonists induced expression of SEAP mRNA (Fig. 3A). Consistent with the results from chemiluminescent assays, *Antrodia camphorata* markedly suppressed induction of SEAP by TCDD. In contrast, induction of SEAP by B[a]P, 3MC and CSE was not attenuated by *Antrodia camphorata*. We also confirmed this result using an endogenous indicator of AhR activation, CYP1A1. As shown in Fig. 3B, all of TCDD, B[a]P, 3MC and CSE induced expression of CYP1A1 mRNA. Consistent with the result of SEAP, *Antrodia camphorata* suppressed induction of CYP1A1 by TCDD, but not by B[a]P, 3MC and CSE. Of note, consistent with the results from the reporter assay (Figs. 1C–E), *Antrodia camphorata* alone weakly induced expression of SEAP and CYP1A1.

Effects of *Antrodia camphorata* on the Expression of Components Involved in the AhR Signaling Pathway The ligand–AhR complex translocates into the nucleus, forms heterodimers with ARNT and binds to DRE, leading to transcriptional induction of target genes.⁶⁾ AhRR is one of the targets for AhR/ARNT, and AhRR induced by AhR/ARNT consequently represses the expression of AhR.⁷⁾ To investigate molecular mechanisms involved in the suppressive effect of *Antrodia camphorata* on the AhR pathway, we examined expression levels of AhR, ARNT and AhRR in the cells treated with *Antrodia camphorata*. Northern blot analysis showed that substantial basal expression of AhR and ARNT was observed in Hepa-1c1c7 cells. In contrast, basal expression of AhRR was not detectable. After the treatment

Fig. 2. Lack of Induction of ER Stress by *Antrodia camphorata*

HeSS8 cells were exposed to 25 pM TCDD in the absence or presence of 10–100 µg/ml *Antrodia camphorata* for 24 h. After the treatment, culture media were subjected to chemiluminescent assay to evaluate SEAP activity and formazan assay, respectively. Activity of SEAP was normalized by the number of viable cells, and relative percentages vs. the value of the cells treated with TCDD alone are shown.

Fig. 3. Effects of *Antrodia camphorata* on AhR-Dependent Gene Expression

HeXS34 cells were exposed to TCDD, B[a]P, 3MC or CSE in the absence or presence of 100 µg/ml *Antrodia camphorata* for 24 h, and expression of SEAP mRNA (A) and CYP1A1 mRNA (B) was evaluated by Northern blot analysis. As a loading control, expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is shown at the bottom. long exp, long exposure.

Fig. 4. Effects of *Antrodia camphorata* on the Expression of Components Involved in the AhR Signaling Pathway

Hepa-1c1c7 cells were treated with serial concentrations of *Antrodia camphorata* in the absence (A) or presence (B) of TCDD for 24 h and subjected to Northern blot analysis of AhR, ARNT and AhRR.

with *Antrodia camphorata*, the expression levels of *AhR* and *ARNT* were not suppressed at any concentrations tested (Fig. 4A). Similarly, expression of *AhRR* was not induced by the treatment with *Antrodia camphorata*. We further performed a similar experiment in the presence of TCDD. We elucidated that the expression levels of *AhR*, *ARNT* and *AhRR* were also unaffected by the treatment with *Antrodia camphorata* even under the stimulation with TCDD (Fig. 4B). Of note, expression of *AhRR* was slightly induced by TCDD, which was not enhanced by *Antrodia camphorata*.

DISCUSSION

Previous investigations suggested that natural products including fruits and vegetables contain biologically active substances that affect activity of AhR. One example is resveratrol (3,5,4'-trihydroxystilbene) contained in grapes and red wine. It is known that resveratrol is an antagonist against dioxin-triggered activation of AhR.²⁸⁾ Vegetable constituents also have the potential for modulating AhR activity.^{29–32)} Some may act as activators of AhR, whereas others are inhibitors of AhR. We previously reported that herbal medicine *Formula bupleuri minor* significantly inhibits activation of AhR by TCDD and that *Glycyrrhizae Radix* and *Scutellariae Radix* are responsible for the suppressive effect.²⁶⁾ However, to date, little is known about the regulatory potential of mycelia on the function of AhR. Recently, we tested 13 mycelial extracts (*Phellinus linteus*, *Cordyceps militaris*, *Lyophyllum decastes*, *Macrolepita gracilentia*, *Naematoloma sublateralitium*, *Agaricus blazei*, *Grifola frondosa*, *Ganoderma lucidum*, *Hericium erinaceum*, *Inonotus obliquus*, *Lentinula edodes*, *Pleurotus nebrodensis*) and found that only *Phellinus linteus* inhibited activation of AhR by TCDD, B[a]P and 3MC.²³⁾ The present report provides another evidence showing the potential of some mycelia to modulate the AhR pathway. Our current result is the first to demonstrate the regulatory potential of *Antrodia camphorata* on the activation of AhR by xenobiotic ligands, especially dioxin.

In this report, we found that *Antrodia camphorata* markedly inhibited activation of AhR and AhR-dependent gene expression by TCDD. In contrast, its effects on PAH- and cigarette smoke-induced activation of AhR were only modest. Furthermore, AhR-dependent gene expression triggered by PAH and cigarette smoke was not obviously affected by *Antrodia camphorata*. It is contrastive to our recent report showing that *Phellinus linteus* suppressed activation of AhR and AhR-dependent gene expression triggered by TCDD, PAH and cigarette smoke.²³⁾ The reason for this discrepancy is currently unclear, but some possibilities may be postulated. We found that *Antrodia camphorata* has the weak potential to induce activation of AhR. It supports an idea that, like α -naphthoflavone,²²⁾ *Antrodia camphorata* is a partial agonist with substantial antagonistic activity. If so, the fact that *Antrodia camphorata* inhibited TCDD-induced AhR activation more efficiently than that by PAH may be simply ascribed to different concentrations of TCDD (25 pM) and PAH (5–15 nM) we used. Indeed, as shown in Fig. 1F, the suppressive effect of *Antrodia camphorata* on the activation of AhR was much weaker when the cells were exposed to 10 nM TCDD. The second possibility is that *Antrodia camphorata* could preferentially interact with TCDD, but not

PAH. In this case, physical binding of TCDD to *Antrodia camphorata* may block the effect of TCDD at the most proximal site of signal transduction. The function of AhR is known to be regulated not only by exogenous ligands but also by endogenous ligands and other factors including co-activators, co-repressors, hormones, cytokines and growth factors.³³⁾ The third possibility is, therefore, that *Antrodia camphorata* and individual AhR agonists might affect levels of those endogenous factors in distinct fashions, leading to different effects on the AhR pathway.

AhR, ARNT and AhRR play crucial roles in the signal transduction pathway triggered by dioxins and PAH. In the present study, we examined expression levels of these molecules in the cells treated with *Antrodia camphorata*. Our results showed that treatment with *Antrodia camphorata* does not affect the levels of *AhR*, *ARNT* and *AhRR* under both TCDD-unstimulated and -stimulated conditions. This result suggests that the suppression of the AhR pathway by *Antrodia camphorata* is independent of down-regulation of AhR/ARNT or up-regulation of AhRR. Together with our finding that the suppressive effect of *Antrodia camphorata* on AhR was much weaker in the cells exposed to a high concentration of TCDD, *Antrodia camphorata* possibly acts as an antagonist of AhR.

Antrodia camphorata has been traditionally used in Taiwan for the treatment of toxication caused by food, alcohol or drugs, as well as for the treatment of diarrhea, hypertension, skin itching and cancer.³⁴⁾ We have experimental evidence showing that *Antrodia camphorata* effectively attenuates alcoholic liver damage in rats (Sugi M. and Matsunaga M., unpublished data). Chemical ingredients found in *Antrodia camphorata* include sesquiterpene lactone, steroids and triterpenoids,^{35–39)} which might be responsible for its therapeutic effects. Our current result indicates the additional beneficial effect of *Antrodia camphorata* for suppression of toxicity caused by some environmental pollutants. Further investigation will be required to identify active entities that block AhR activation.

Acknowledgments We thank Dr. Yoshiaki Fujii-Kuriyama (University of Tsukuba) for providing us with cDNAs for ARNT and AhRR, Dr. Kazuhiro Sogawa (Tohoku University) for AhR cDNA and Dr. Hideaki Kikuchi (Hiroshima University) for CYP1A1 cDNA. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 16390243, No. 19651024 and No. 17651026 to M. K.).

REFERENCES

- 1) Birnbaum L. S., Tuomisto J., *Food Addit. Contam.*, **17**, 275–288 (2000).
- 2) Tohyama C., *Environ. Sci.*, **9**, 37–50 (2003).
- 3) Kasai A., Hiramatsu N., Hayakawa K., Yao J., Maeda S., Kitamura M., *Cancer Res.*, **66**, 7143–7150 (2006).
- 4) Kasai A., Hiramatsu N., Hayakawa K., Yao J., Kitamura M., *Environ. Health Perspect.*, **116**, 349–354 (2008).
- 5) Kitamura M., Kasai A., *Cancer Lett.*, **252**, 184–194 (2007).
- 6) Denison M. S., Pandini A., Nagy S. R., Baldwin E. P., Bonati L., *Chem. Biol. Interact.*, **141**, 3–24 (2002).
- 7) Mimura J., Ema M., Sogawa K., Fujii-Kuriyama Y., *Genes Dev.*, **13**, 20–25 (1999).

- 8) Fernandez-Salguero P. M., Hilbert D. M., Rudikoff S., Ward J. M., Gonzalez F. J., *Toxicol. Appl. Pharmacol.*, **140**, 173—179 (1996).
- 9) Mimura J., Yamashita K., Nakamura K., Morita M., Takagi T. N., Nakao K., Ema M., Sogawa K., Yasuda M., Katsuki M., Fujii-Kuriyama Y., *Genes Cells*, **2**, 645—654 (1997).
- 10) Shimizu Y., Nakatsuru Y., Ichinose M., Takahashi Y., Kume H., Mimura J., Fujii-Kuriyama Y., Ishikawa T., *Proc. Natl. Acad. Sci. U.S.A.*, **97**, 779—782 (2000).
- 11) Andersson P., McGuire J., Rubio C., Gradin K., Whitelaw M. L., Pettersson S., Hanberg A., Poellinger L., *Proc. Natl. Acad. Sci. U.S.A.*, **99**, 9990—9995 (2002).
- 12) Andersson P., Ridderstad A., McGuire J., Pettersson S., Poellinger L., Hanberg A., *Biochem. Biophys. Res. Commun.*, **302**, 336—341 (2003).
- 13) Nohara K., Pan X., Tsukumo S., Hida A., Ito T., Nagai H., Inouye K., Motohashi H., Yamamoto M., Fujii-Kuriyama Y., Tohyama C., *J. Immunol.*, **174**, 2770—2777 (2005).
- 14) Tauchi M., Hida A., Negishi T., Katsuoka F., Noda S., Mimura J., Hosoya T., Yanaka A., Aburatani H., Fujii-Kuriyama Y., Motohashi H., Yamamoto M., *Mol. Cell. Biol.*, **25**, 9360—9368 (2005).
- 15) Borchers A. T., Krishnamurthy A., Keen C. L., Meyers F. J., Gershwin M. E., *Exp. Biol. Med.* (Maywood), **233**, 259—276 (2008).
- 16) Yang H. L., Chen C. S., Chang W. H., Lu F. J., Lai Y. C., Chen C. C., Hseu T. H., Kuo C. T., Hseu Y. C., *Cancer Lett.*, **231**, 215—227 (2006).
- 17) Kuo M. C., Chang C. Y., Cheng T. L., Wu M. J., *J. Ethnopharmacol.*, **120**, 196—203 (2008).
- 18) Chen J. J., Lin W. J., Liao C. H., Shieh P. C., *J. Nat. Prod.*, **70**, 989—992 (2007).
- 19) Kasai A., Hiramatsu N., Meng Y., Yao J., Takeda M., Maeda S., Kitamura M., *Anal. Biochem.*, **335**, 73—80 (2004).
- 20) Kasai A., Hiramatsu N., Meng Y., Yao J., Maeda S., Kitamura M., *Anal. Biochem.*, **337**, 84—88 (2005).
- 21) Tagawa Y., Hiramatsu N., Kasai A., Hayakawa K., Okamura M., Yao J., Kitamura M., *Free Radic. Biol. Med.*, **45**, 50—59 (2008).
- 22) Kasai A., Yao J., Yamauchi K., Hiramatsu N., Hayakawa K., Meng Y., Maeda S., Kitamura M., *Toxicol. Appl. Pharmacol.*, **211**, 11—19 (2006).
- 23) Mukai M., Kasai A., Hiramatsu N., Hayakawa K., Okamura M., Tagawa Y., Yao J., Nakamura T., Kitamura M., *Biol. Pharm. Bull.*, **31**, 1888—1893 (2008).
- 24) Sogawa K., Numayama-Tsuruta K., Takahashi T., Matsushita N., Miura C., Nikawa J., Gotoh O., Kikuchi Y., Fujii-Kuriyama Y., *Biochem. Biophys. Res. Commun.*, **318**, 746—55 (2004).
- 25) Shibasaki M., Takeuchi T., Ahmed S., Kikuchi H., *J. Biol. Chem.*, **279**, 3869—3876 (2004).
- 26) Kasai A., Hiramatsu N., Hayakawa K., Yao J., Kitamura M., *Biol. Pharm. Bull.*, **31**, 838—846 (2008).
- 27) Hiramatsu N., Kasai A., Hayakawa K., Yao J., Kitamura M., *Nucleic Acids Res.*, **34**, e93 (2006).
- 28) Casper R. F., Quesne M., Rogers I. M., Shiota T., Jolivet A., Milgrom E., Savouret J. F., *Mol. Pharmacol.*, **56**, 784—790 (1999).
- 29) Zhang S., Qin C., Safe S. H., *Environ. Health Perspect.*, **111**, 1877—1882 (2003).
- 30) Amakura Y., Tsutsumi T., Nakamura M., Kitagawa H., Fujino J., Sasaki K., Toyoda M., Yoshida T., Maitani T., *Biol. Pharm. Bull.*, **26**, 532—539 (2003).
- 31) Ashida H., *Biofactors*, **12**, 201—206 (2000).
- 32) Amakura Y., Tsutsumi T., Sasaki K., Yoshida T., Maitani T., *Biol. Pharm. Bull.*, **26**, 1754—1760 (2003).
- 33) Harper P. A., Riddick D. S., Okey A. B., *Biochem. Pharmacol.*, **72**, 267—279 (2006).
- 34) Tsai Z., Liaw S., “The Use and the Effect of Ganoderma,” Sheng-Yun Press, Taichung, Taiwan, 1985, pp. 116—117.
- 35) Chen C., Yang S., Shen Y., *J. Nat. Prod.*, **58**, 1655—1661 (1995).
- 36) Cherng I., Chiang H., *J. Nat. Prod.*, **58**, 365—371 (1995).
- 37) Cherng I., Wu D., Chiang H., *Phytochemistry*, **41**, 263—267 (1996).
- 38) Chiang H., Wu D., Cherng I., Ueng C., *Phytochemistry*, **39**, 613—616 (1995).
- 39) Yang S., Shen Y., Chen C., *Phytochemistry*, **41**, 1389—1392 (1996).