FEBS Letters 583 (2009) 2377–2384

FEBS Letters

journal homepage: www.FEBSLetters.org

Expression profiling of the genes responding to zearalenone and its analogues using estrogen-responsive genes

Meher Parveen, Yun Zhu, Ryoiti Kiyama *

Signaling Molecules Research Group, Neuroscience Research Institute, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan

ARTICLE INFO

Article history:
Received 22 May 2009
Revised 18 June 2009
Accepted 18 June 2009
Available online 23 June 2009

Edited by Ned Mantei

Keywords:
DNA microarray
Gene expression profile
Cell signaling
Gene function
Estrogen

ABSTRACT

To compare gene expression profiles in response to estrogen or 17β-estradiol (E_2) and a mycotoxin, zearalenone (ZEA), and its analogues (collectively termed ZEA compounds), breast cancer MCF-7 cells were treated with 10 nM of E_2 or ZEA compounds including ZEA, α-zearalenol, β-zearalenol, zearalanone, α-zearalanol and β-zearalanol. Expression profiles for 120 estrogen-responsive genes were subjected to cluster and statistical analyses using correlation coefficients or *R*-values. We found that all of the ZEA compounds stimulated the growth of MCF-7 cells, as much as E_2, and showed similar expression profiles to that of E_2 (*R*-values ranged from 0.82 to 0.96). The effect of ZEA compounds was likely mediated by estrogen-receptor-dependent Erk1/2-signaling. These results provide clues to understand the mechanism of their estrogen-like action.

1. Introduction

Zearalenone (ZEA), also known as F-2 toxin, is a non-steroidal estrogenic mycotoxin produced by various species of *Fusarium* [1,2]. ZEA and its analogues constitute an important class of endocrine disruptors, which have estrogenic effects and influence reproduction, such as 17β-estradiol (E_2) [3]. In fact, ZEA and its metabolites were found to bind to estrogen receptors [4,5]. Several lines of evidence show that ZEA is associated with hyperestrogenism and physiological alterations of the reproductive tract [6], affects conception, implantation and fetal development [7], and also disturbs the ovulation cycle and reduces the body sizes of domestic animals, particularly swine [8] and rats [9].

Toxic effects of ZEA were demonstrated at the cellular and molecular levels, such as the induction of apoptosis, DNA fragmentation [10], production of micronuclei [11], chromosomal aberrations [12], and formation of DNA adducts [13]. All these toxic effects are unlikely to be due only to the estrogenic activity of ZEA, and other toxic effects, not related to the affinity for estrogen receptors, could be involved. Several processes are known to play roles in the molecular events leading to cell damage, particularly inhibition of the synthesis of cellular macromolecules and induction of lipid peroxidation. Lipid peroxidation is one of the cellular pathways involved in oxidative damage and underlies ZEA-induced apoptosis [14].

Anti-apoptotic effects of ZEA at low concentrations were reported. The inhibition of apoptosis by ZEA was correlated with altered expression levels of the apoptosis-related modulators *bax* and *bcl-2*. Thus, the anti-apoptotic action of ZEA in MCF-7 cells was closely linked with the down-regulation of *bax* expression, which is different from its behavior in the anti-apoptotic effect of E_2, and up-regulation of *bcl-2* expression [15]. Therefore, the estrogenic activity of ZEA and the signaling pathway involved is not completely the same as that of E_2, and there may be specific types of modulation of the estrogenic signal for each of ZEA compounds. To analyze the differences in the effect of estrogenic chemicals on gene expression, human breast cancer cells or mouse uterus were treated with E_2, phytoestrogens or xenoestrgens, and global gene expression profiles were analyzed by using DNA microarrays [16–18]. The expression profiles may depend on the subtypes of estrogen receptors [18].

In this study, we tried to evaluate the estrogenecity of ZEA and its analogues at the molecular level by using a focused microarray. DNA microarrays have opened a new paradigm in toxicology [19], by characterizing the genome-wide response of gene expression stimulated by endocrine-disrupting chemicals and by offering a means of understanding the biological effects and mechanisms of

Abbreviations: E_2, 17β-estradiol; ZEA, zearalenone; ZAL, zearalanone; DCC-FBS, dextran-coated charcoal-treated fetal bovine serum; SRB, sulforhodamine B

* Corresponding author. Fax: +81 298616190.
E-mail address: kiyama.r@aist.go.jp (R. Kiyama).

0014-5793/$36.00 © 2009 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2009.06.035

estrogenicity on a genome-wide scale. We have already applied this technology to several chemicals of artificial and natural origins [20–24]. Here, we obtained expression profiles of estrogen-responsive genes for ZEA and its analogues and attempted to identify specific genes or biological pathways to understand their mode of action.

2. Materials and methods

2.1. Chemicals, cell culture and RNA isolation

Natural estrogen E_2, and ZEA and its derivatives were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Sigma–Aldrich (St. Louis, MO), respectively. All the test compounds were dissolved in dimethylsulfoxide with the final concentration of the solvent not more than 0.1% of the culture medium. MCF-7 cells were obtained from the Japanese Collection of Research Biosources Cell Bank (National Institute of Health Science, Tokyo, Japan). Cells were maintained in phenol red-free RPMI 1640 medium (Invitrogen) containing 10% dextran-coated charcoal-treated fetal bovine serum (DCC-FBS) and incubated at 37 °C in an atmosphere of 5% CO_2–95% air for 3 days. E_2 and ZEA compounds were added individually to the medium and the final concentration for each was 10 nM. After incubation, total RNA was isolated using Isogen (Wako Pure Chemical Industries) according to the manufacturer's instructions and quantified by measuring optical density at 260 nm.

2.2. Sulforhodamine B assay

The Sulforhodamine B (SRB) assay for measuring cell proliferation was performed according to Skehan et al. [25] with some modifications as follows. Cells (10^4 cells/ml) were seeded on 24-well plates with DCC-FBS medium for 3 days at 37 °C. The cultures were then incubated in the presence of 10 nM E_2 or ZEA (0.01 nM, 0.1 nM, 1 nM, 10 nM, 100 nM, 1 μM, or 10 μM) for 3 more days. Next, the cells were fixed in 10% cold trichloroacetic acid at 4 °C for 30 min, stained with 200 μl of 0.4% SRB, and dissolved in 1% acetic acid for 20 min. A 200-μl volume of 10 nM unbuffered tris-base was used for the solubilization of bound protein. Solutions were transferred into 96-well plates to measure optical density at 490 nm. The background level was subtracted by measuring optical density at 650 nm.

2.3. cDNA microarray and data analysis

A customized cDNA microarray (EstrArray; InfoGenes, Tsukuba, Japan) was used as described [20]. It was manufactured by mechanical spotting of cDNA (approximately 0.5–1.5 kb) on glass slides containing 172 estrogen-responsive genes (108 up-regulated and 64 down-regulated genes). In addition, it contains extra 31 expression/calibration markers. However, for the present study, we have chosen 120 estrogen-responsive genes with greater statistical stability, from among the original 172 genes [22]. The EstrArray assay was performed in triplicate using independently prepared sets of total RNA. Labeling of cDNA probes, hybridization on EstrArray, signal detection and data analyses were done as described previously [22]. The ratios of Cy3- and Cy5-signal intensities (Cy3/Cy5) were calculated and $\log_2$-transformed. Then, $\log_2$ (Cy3/Cy5) values were normalized against an average of 28 internal control genes. Thus obtained $\log_2$ (Cy3/Cy5) values from two spots on the microarray were averaged and used for further analyses. Average-linkage hierarchical clustering was performed using the Cluster program [26] and the results were displayed with the Tree View program [26]. Coefficients of correlations between gene expression profiles and *p*-values were calculated using SPSS 12.0J (SPSS Japan; Tokyo, Japan). The DNA microarray data discussed here have been deposited in NCBI's Gene Expression Omnibus [27] and are accessible through the Accession Number GSE15249 (http://www.ncbi.nlm.nih.gov/geo/).

The UniGene names of the 120 genes analyzed are based on the Entrez Gene database (www.ncbi.nlm.nih.gov). The categories and gene functions for classification were based on the Gene Ontology terms in the Entrez Gene database. If there were several functions, only one was used as representative for each gene.

2.4. Real-time quantitative reverse transcription-PCR

Total RNA was extracted from MCF-7 cells as described previously [20]. First strand cDNA was synthesized from 1 μg of total RNA using a SuperScript III Platinum Two-Step qRT-PCR kit (Invitrogen). Quantitative real-time PCR was performed with a LightCycler (Roche; Basel, Switzerland) using Platinum SYBR-Green (Invitrogen) for detection. The denaturing of cDNA at 95 °C for 2 min was followed by 45 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 5 s and extension at 72 °C for 20 s. β-Actin was used as a control. The primer sequences are: *TFF1*, 5′-TTGTGGTTTTCCTGGTGTCA-3′ and 5′-CCGAGCTCTGGGACTAATCA-3′; *SH3BP5*, 5′-AAAGAACCAGAGCTGGGAAGATG-3′ and 5′-ATCGTGGGATAAAGTGGAGAGGA-3′; *AGTR1*, 5′-CCTGGCTATTGTTCACCCAAT-3′ and 5′-GGGACTCATAATGGAAAGCACA-3′; *ARGHDIA*, 5′-CCTCACTAGCCTCTACTCCCTGT-3′ and 5′-ACTGAGGTGACTTGAGTGTTGG-3′ actin, 5′-CTGGAACGGTGAAGGTGACA-3′ and 5′-AAGGGACTTCCTGTAACAATGCA-3′. The data were normalized as a ratio to the control and expressed as the $\log_2$-transformed fold-change in mRNA relative to that before chemical treatment.

2.5. Western blotting

The protein was electro-transferred onto nitrocellulose membranes (Millipore; Billerica, MA) using a semi-dry transfer cell (BIO-RAD; Hercules, CA) at 1 mA/cm^2 for 2 h. The membranes were soaked in Tris-buffered saline containing 0.1% Tween 20 and 5% BSA (TBST-BSA), and then probed overnight at 4 °C with a phospho-Erk1/2 antibody (Cell Signaling Technologies; used after a 1:1000 dilution in TBST-BSA), which detects phosphorylated forms of Erk1/2. After being washed with TBST, the membranes were incubated in TBST-BSA containing a horseradish peroxidase-conjugated goat antibody against rabbit IgG (Cell Signaling Technologies) for 1 h and then visualized with the ECL-plus Western Blotting Detection System (Amersham Pharmacia Biotech, Arlington Heights, IL) using Cool Saver AE-6955 (ATTO; Tokyo, Japan). After stripping, the same blot was re-probed with an anti-total Erk1/2 antibody (Cell Signaling Technologies) to the relative level of total Erk1/2 protein. The intensity of the bands was quantified by the Multi Gauge Ver 3.0 software (FUJIFILM). For statistical evaluation of the data, *p*-values were calculated using SPSS 12.0J.

3. Results

3.1. Cell proliferation assay with ZEA compounds

First, we examined the effects of ZEA compounds on cell proliferation. We used the SRB assay to examine the proliferation of MCF-7 cells treated with either ZEA, α-ZEA, β-ZEA, ZAL, α-ZAL, or β-ZAL, or with natural estrogen (E_2) as a control (Fig. 1A). All the ZEA compounds induced cell proliferation at relatively low concentrations, comparable to that of E_2 (Fig. 1B–G), confirming their estrogenicity as reported [4,5]. Furthermore, all except α-ZEA suppressed cell proliferation at 10 μM. On the other hand, α-ZEA induced cell proliferation over a broad range of concentrations, from 0.01 nM to 10 μM.

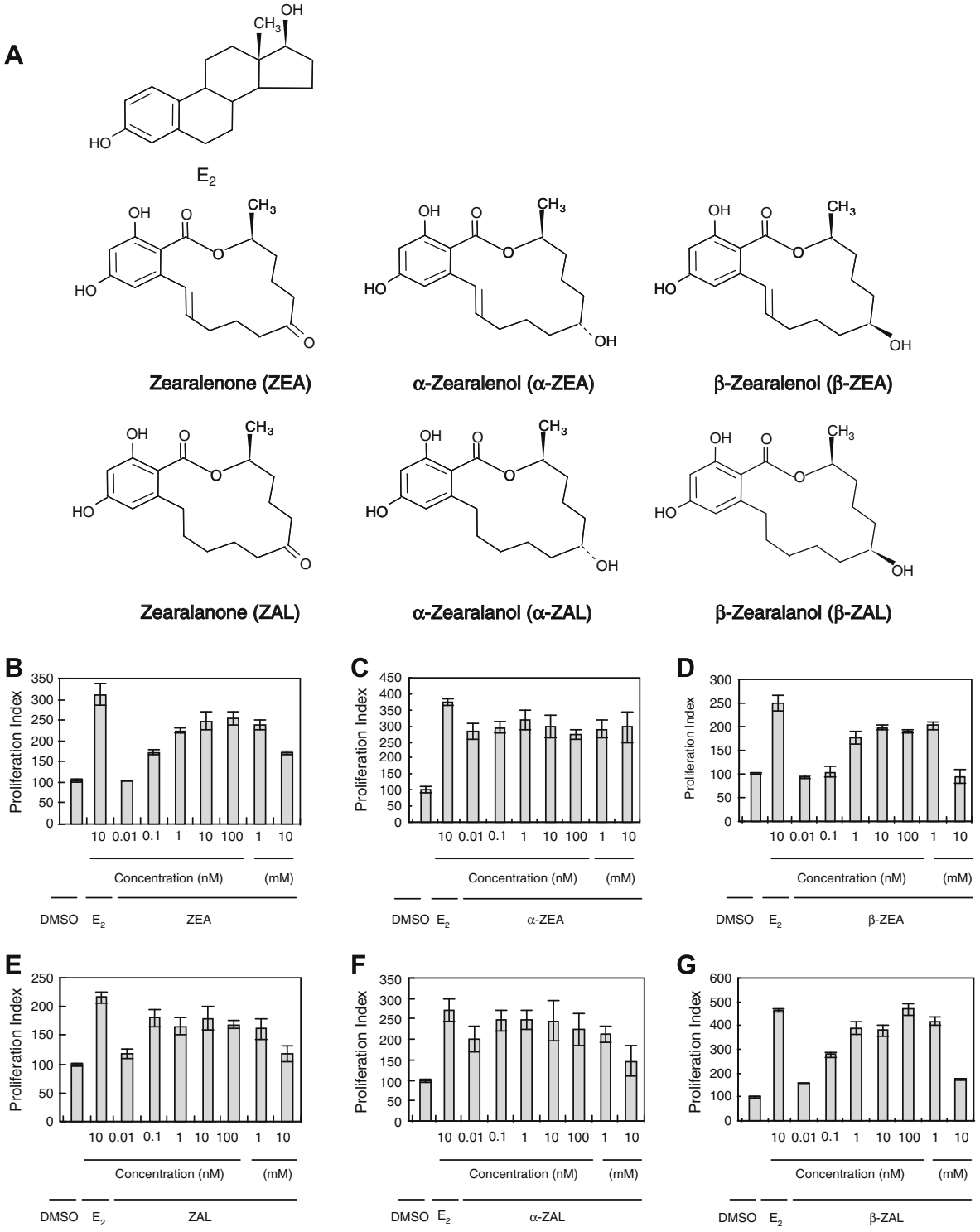

Fig. 1. Chemical structure and effects on cell proliferation of E_2 and ZEA compounds. (A) Chemical structure of E_2 and ZEA compounds. (B–G) Cell proliferation assay for ZEA (B), α-ZEA (C), β-ZEA (D), ZAL (E), α-ZAL (F) and β-ZAL (G). MCF-7 cells were treated with vehicle (DMSO), E_2 (10 nM) or various concentrations of ZEA compounds as indicated, and cultured for 72 h. The effect on cell proliferation was examined by sulforhodamine B assay. Rates of cell proliferation in response to E_2 or ZEA compounds are shown relative (%) to the control (Proliferation Index). Each experiment was repeated four times and the average and S.D. are shown.

Based on these results, we used a fixed concentration of 10 nM for all ZEA compounds in the DNA microarray assay (Fig. 2).

3.2. Expression profiles for ZEA compounds in MCF-7 cells

We then examined expression profiles of estrogen-responsive genes using a customized DNA microarray (Fig. 2). A set of 120 highly reproducible estrogen-responsive genes [22] was used for the assay and the data were subjected to a correlation analysis based on linear regression, with correlation coefficients or *R*-values used to compare gene expression profiles (Fig. 2). First, the profiles for these ZEA compounds were compared with that of E_2 (Fig. 2). *R*-values ranged from 0.93 to 0.96 for ZEA compounds, except for β-ZEA, which showed an *R*-value of 0.82 (Fig. 2A–F). On the other

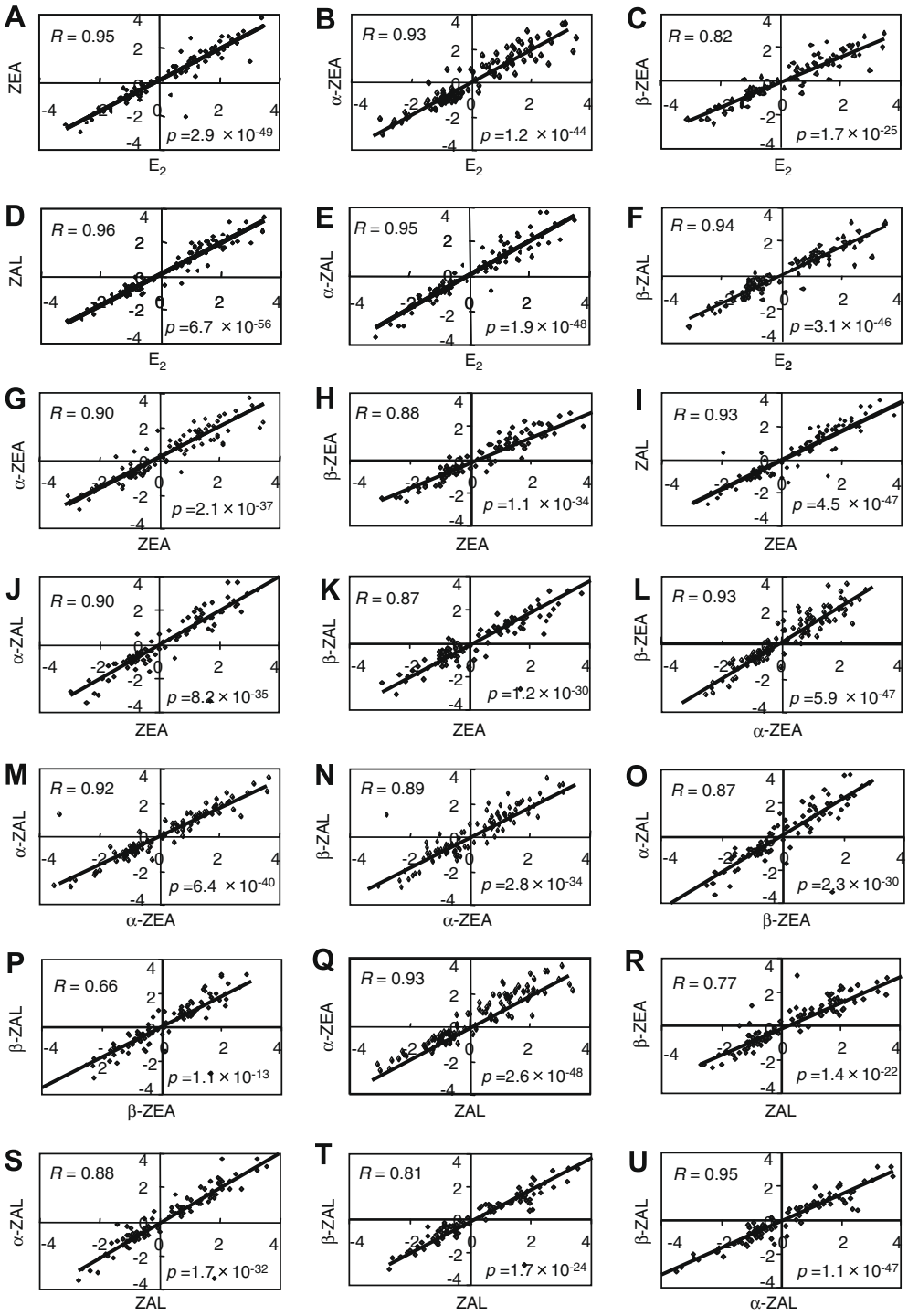

Fig. 2. Correlation of gene expression profiles between respective pairs of E_2 and ZEA compounds. Expression profiles of 120 estrogen-responsive genes were compared among E_2 and the ZEA compounds; zearalenone (ZEA), α-zearalenol (α-ZEA), β-zearalenol (β-ZEA), zearalanone (ZAL), α-zearalanol (α-ZAL) and β-zearalanol (β-ZAL). The axes give $\log_2$-transformed ratios of the fluorescent intensity with the chemical to that without the chemical (vehicle). Coefficients (*R*-values) of correlations between two profiles and *p*-values for evaluating the statistical significance of the correlation were calculated in each graph on the basis of linear regression.

hand, a wide range of *R*-values (0.66 to 0.95) were found for the expression profiles between pairs of ZEA compounds. The pair α-ZAL and β-ZAL showed the highest *R*-value (0.95) (Fig. 2U) and the pair β-ZEA and β-ZAL the lowest (0.66) (Fig. 2P), which suggests that some genes are regulated in different ways in response to different ZEA compounds. The expression profiles were also subjected to a cluster analysis (Fig. 3A). The results indicate that E_2 and all the ZEA compounds examined here had very similar expression profiles and E_2 was closer to ZAL, α-ZEA and ZEA than the others.

3.3. Different effects on signaling-related genes by ZEA compounds

The genes used in our microarray assay can be categorized into six broad groups (enzymes, signaling, proliferation, transcription, transport and others) based on their known functions ([22]; see Fig. 3A). To understand functional differences among the genes in response to different ZEA compounds, we analyzed gene expression profiles by comparing *R*-values for each functional group. We first compared the expression profiles for E_2 with those for

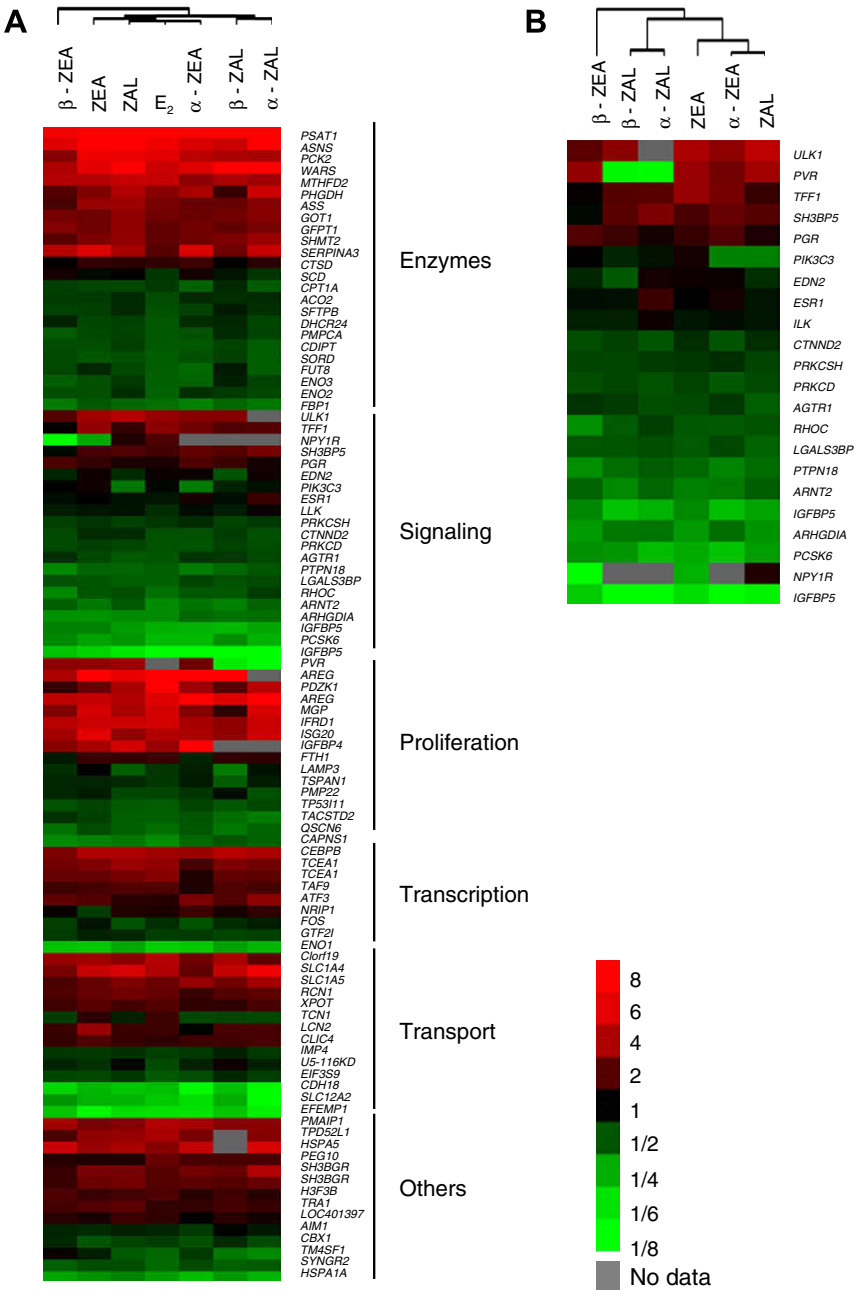

Fig. 3. Functional cluster analysis. (A) ZEA compounds were clustered according to the expression profiles using a set of 120 genes. Up- and down-regulated genes in the profiles are presented as red and green, respectively, and a color scale indicates the ratio of the signal for each chemical (Cy3 signal) to that for vehicle (Cy5 signal). Estrogen-responsive genes were categorized into six functional groups based on Gene Ontology available in the Entrez database. (B) Genes belonging to signaling group for each ZEA compound are clustered as described in panel A. The profile for E_2 was omitted here to compare the relationship among ZEA compounds.

different ZEA compounds, and between ZEA compounds (data not shown). The results indicate that E_2 and ZEA compounds are quite similar. *R*-values of all functional groups except the signaling-related genes were again very similar among the ZEA compounds.

The signaling-related genes examined here totaled 20 and their expression profiles as a whole clearly showed differences among three groups in the cluster analysis (Fig. 3B); the group of β-ZEA, the group of α-ZAL and β-ZAL, and the rest. Obviously, these relationships could be caused by differences in the response of certain genes. The signaling-related genes used here can be further classified according to signaling pathways, such as mitogen-activated protein kinase- (MAPK-) related genes (*SH3BP5* for example), Akt2-related genes (*PRKCD* for example) and Ras superfamily genes (*RHOC* and *ARHGDIA* for example). We examined the expression levels of some signaling-related genes by real-time RT-PCR (Fig. 4). The results of four genes showing up-regulation (*TFF1* and *SH3BP5*; Fig. 4E and F) or down-regulation (*AGTR1*, and *ARHGDIA*; Fig. 4G and H) clearly validated the results of the microarray assay (Fig. 4A–D).

3.4. Activation of Erk1/2 in MCF-7 cells by treatment with ZEA compounds

External growth factors can activate the rapid signaling pathway within an hour, eventually inducing cell proliferation [28]. Here, we examined whether ZEA compounds can activate the extracellular-signal-regulated kinase 1/2 (Erk1/2) to induce cell proliferation. The levels of the phosphorylated forms and total

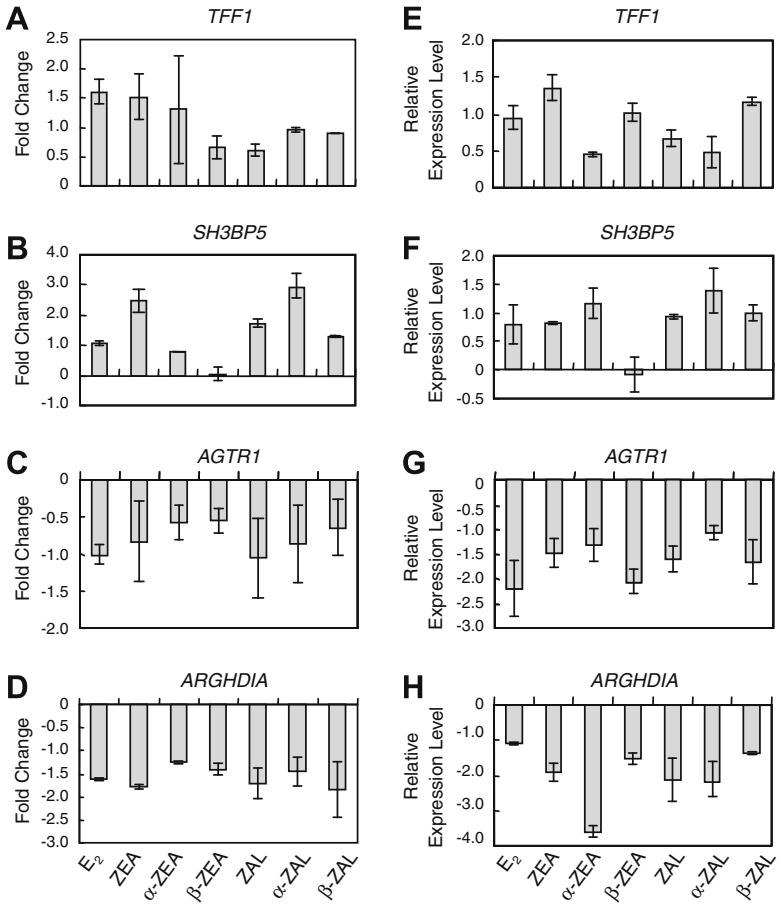

Fig. 4. Validation of microarray results by real-time RT-PCR. Microarray data (A–D) for four genes (*TFF1*, *SH3BP5*, *AGTR1* and *ARGHDIA*) were compared with the results obtained by quantitative real-time RT-PCR (E–H). RNA (1 μg) was used for cDNA synthesis. Each experiment was repeated three times and the average and S.D. are shown. All data shown are $\log_2$-transformed values.

protein were quantified after the treatment of MCF-7 cells with 10 nM of ZEA compounds and the results compared with those for E_2 at the same concentration (Fig. 5). Phosphorylation of Erk1/2 was clearly observed in response to all ZEA compounds examined (Fig. 5A). To further substantiate this observation, we pretreated the cells with ICI 182 780 and examined whether it blocks the phosphorylation. Consistent with a previous study [23], phosphorylation of Erk1/2 was inhibited in E_2-treated cells by the pretreatment with ICI 182 780 (Fig. 5B and C). Also, the pretreatment with ICI 182 780 resulted in the inhibition of phospho-Erk1/2 (P-Erk) in the cells treated with ZEA compounds. This observation clearly suggests that the signaling pathway was very similar between E_2 and the ZEA compounds.

4. Discussion

4.1. Estrogenic activity of ZEA compounds

It is believed that ZEA compounds can attain an E_2-like conformation very easily. This structural flexibility enables them to bind ER as strongly as natural estrogen [29]. Consistent with this, we observed that ZEA compounds induced strong estrogenic signals at very low concentrations, as low as 0.01 nM. This observation is quite consistent with a previous study in which various ZEA compounds were found to stimulate the proliferation of MCF-7 cells at the equivalent concentrations [30]. On the basis of EC_{50} values, ZEA compounds were ranked as follows: α-ZEA = α-ZAL = β-ZAL = ZEA > β-ZEA [30]. Similarly, we also observed strong estrogenic activity for α-ZEA and α-ZAL and weak activity for β-ZEA (Fig. 1C, F and D) and could be ranked as follows: α-ZEA = α-ZAL > ZAL > ZEA = β-ZAL > β-ZEA. Consistent with this observation, when gene expression profiles were compared, all of the ZEA compounds except β-ZEA showed high correlation coefficients with E_2. The cluster analysis of gene expression profiles also categorized β-ZEA further away from E_2 than other compounds. Collectively, it might suggest that α-ZEA could be structurally closer to natural estrogen than β-ZEA.

4.2. Different effects on genes by ZEA compounds

Among the functional groups of the genes examined here, the genes related to signaling contributed to significant differences in the expression profiles among three subgroups of ZEA compounds; β-ZEA alone, α-ZAL and β-ZAL, and ZEA, α-ZEA and ZAL (Fig. 3B). We further examined the genes contributing to this difference by ANOVA and *t*-test but could not find genes independently showing differences at significant levels ($p < 0.05$) except *PVR* and *IGFBP5* (data not shown). A part of the reason for this is that the degree of expressional difference for these genes is not very high, although sets of genes, probably grouped by more specific functions, could collectively show more significant differences. Furthermore, the functions of many of the signaling-related genes remain unclear. *PVR* has been recognized as a poliovirus receptor but its intracellular signaling is not known. Down-regulation of *IGFBP5*, a growth inhibitor and pro-apoptotic agent in breast cancer cells [31], would result in activation of cell proliferation as observed here, although the mechanism is not well studied. On the other hand, there are several classes of genes; *Ras*-related genes, *ULK1*, *RHOC* and *ARHG-*

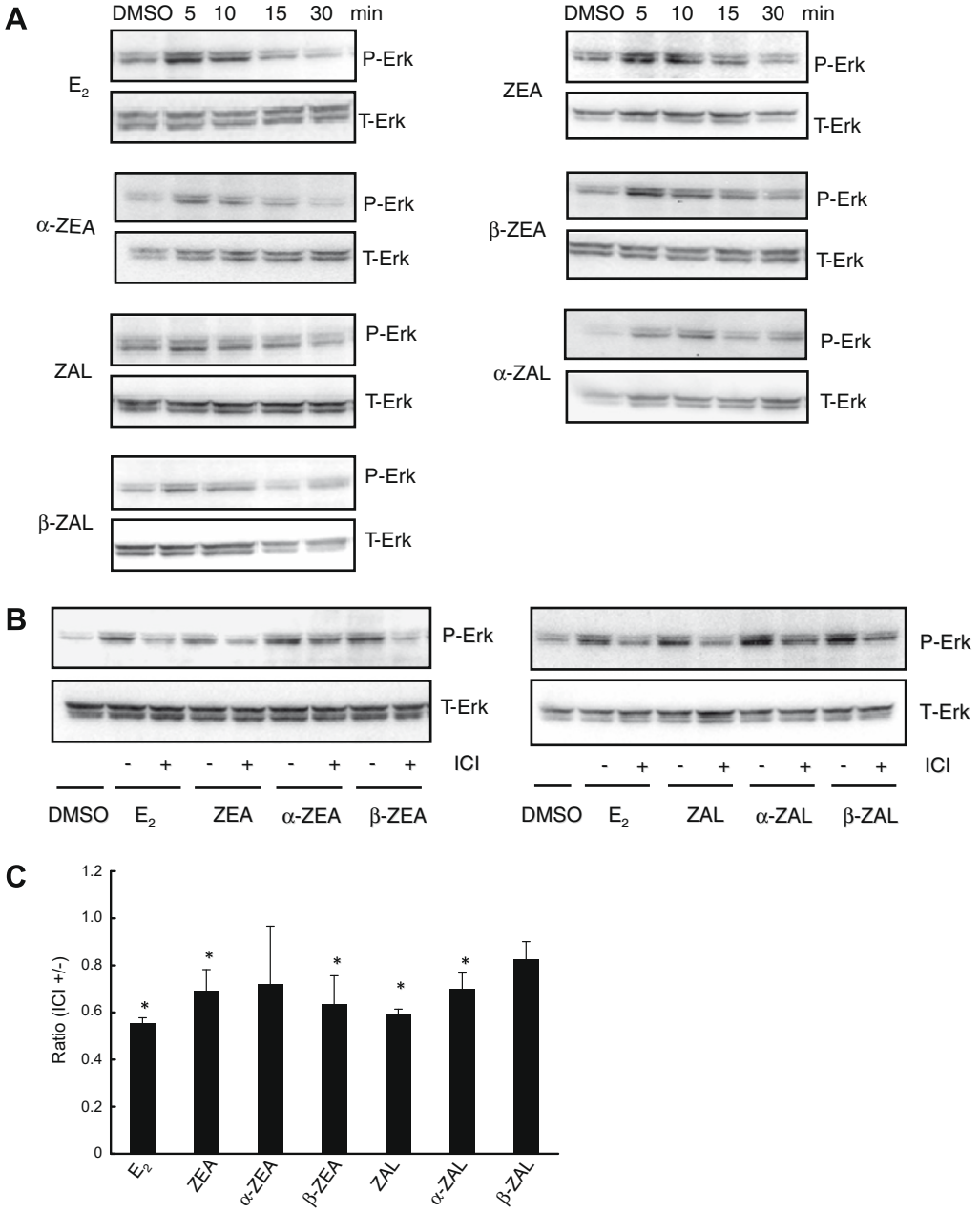

Fig. 5. Activation of Erk1/2 by ZEA compounds. (A) Time course of phosphorylation. Cells were treated with ZEA compounds for the periods as indicated. (B) Inhibition of the phosphorylation of Erk1/2 with ICI 182 780. Cells were treated with 1 μM ICI 182 780 (ICI) for 60 min prior to their incubation with E_2 or ZEA compounds for 5 min. The level of P-Erk was examined by Western blot analysis. Cells were lysed in SDS sample buffer and protein samples were electrophoresed in SDS–15% polyacrylamide gels, transferred to nitrocellulose membranes and probed with an antibody specific to phospho-Erk1/2 (P-Erk). The level of protein loading was confirmed with an antibody that recognizes total Erk1/2 (T-Erk) protein. (C) Quantitative analysis of Western blot data for the inhibition of the phosphorylation of Erk1/2 with ICI 182 780. The intensity of the band for each chemical was normalized with that of the control (DMSO), and the average and S.D. of the ratios (ICI +/−) for each chemical after repeating the assay three times (including the data shown in panel B) are shown. Statistical significance of the deviation of the response to each chemical from no change (i.e. ICI +/− = 1.0) was analyzed by *t*-test. $^{*}p < 0.05$.

DIA; receptors and their ligands, *NPY1R*, *PGR*, *AGTR1*, *ARNT2* and *PVR*; kinases/phosphatases and their binding proteins, *ULK1*, *SH3BP5*, *PI3K3C3*, *ILK*, *PRKCSH*, *PRKCD* and *PTPN18*. These genes could activate specific Ras proteins and other signaling proteins thereby contributing to cell growth and proliferation. Down-regulation of apoptosis-related genes, *PRKCD* and *IGFBP5*, would be a part of this.

4.3. ZEA compounds and signaling

Previous studies showed that the pathway including Erk1/2 phosphorylation mediates the estrogen-like signal for cell proliferation induced by ginsenoside Rg1, genistein or equol in MCF-7 cells [32,33]. The inhibition of Erk1/2 phosphorylation was shown to cause the reduced expression of estrogen-responsive genes, such as *pS2* [32]. In this study, we found that ZEA compounds can trigger a rapid activation of the Erk1/2 pathway within 5 min. The pattern of signal activation of MCF-7 cells with ZEA compounds is similar to that in response to E_2. However, the underlying mechanism of E_2-mediated activation of Erk1/2 is not well understood. Reports in the last decade proposed two models to explain E_2-mediated non-genomic rapid cell proliferation. One model suggests that membrane-bound classic ER α/β together with other factors mediates the E_2-induced signaling [34,35]. Meanwhile, the other

model indicates the involvement of another membrane-bound G-protein-coupled protein, GPR30, in E_2-mediated estrogenicity [36,37]. The inhibition of Erk1/2 phosphorylation with ICI 182 780 in the cells treated with ZEA compounds (Fig. 5) indicates that membrane-bound ER α/β could be associated with the estrogenic action mediated by ZEA compounds. On the other hand, a delayed activation of Erk1/2 is involved in cell proliferation induced by genistein or equol in an ICI 182 780-independent manner [32]. Therefore, Erk1/2 phosphorylation should be involved in different receptors and their downstream signaling pathways with proper timing and partners for its actions.

Acknowledgements

This work was supported by a grant for supporting small and medium enterprises from the Ministry of Economy, Trade and Industry, and a Grant-in-Aid for Basic Areas and a JSPS fellowship from the Ministry of Education, Science, Sports and Culture of Japan. M.P. was selected as a JSPS fellow and a trainee in the advanced technology program of the Japan Industrial Technology Association (JITA).

References

[1] Hidy, P.H., Baldwin, R.S., Greasham, R.L., Keith, C.L. and McMullen, J. (1977) Zearalenone and some derivatives: production and biological activities. Adv. Appl. Microbiol. 22, 59–82.
[2] Tanaka, T., Hasegawa, A., Yamamoto, S., Lee, U.S., Sugiura, Y. and Ueno, Y. (1988) Worldwide contamination of cereals by the Fusarium mycotoxins, nivalenol, deoxynivalenol and zearalenone. 1. Survey of 19 countries. J. Agric. Food Chem. 36, 979–983.
[3] Diekman, M.A. and Green, M.L. (1992) Mycotoxins and reproduction in domestic livestock. J. Anim. Sci. 70, 1615–1627.
[4] Katzenellenbogen, B.S., Katzenellenbogen, J.A. and Mordecai, D. (1979) Zearalenones: characterization of the estrogenic potencies and receptor interactions of a series of fungal beta-resorcylic acid lactones. Endocrinology 105, 33–40.
[5] Miksicek, R.J. (1994) Interaction of naturally occurring nonsteroidal estrogens with expressed recombinant human estrogen receptor. J. Steroid Biochem. Mol. Biol. 49, 153–160.
[6] Creppy, E.E. (2002) Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicol. Lett. 127, 19–28.
[7] Long, G.G. and Diekman, M.A. (1989) Effect of zearalenone on early pregnancy in guinea pigs. Am. J. Vet. Res. 50, 1220–1223.
[8] Osweiler, G.D., Carson, T.L., Buck, W.B. and Van Gelder, G.A. (1985) Biotoxins. Clinical, and Diagnostic Veterinary Toxicology, third ed, Kendall/Hunt Publishing Company, Dubuque, IA. pp. 403–452.
[9] Bacha, H., Chekir, L., Ellouz, F., Hadidane, R. and Creppy, E.E. (1993) Effects of zearalenone on fertilisation and gestation in rats in: Proceedings of the UK Workshop, Occurrence and Significance of Mycotoxin (Scudamore, K.A., Ed.), pp. 258–262, The University of West London, Central Sciences Laboratory, London.
[10] Kim, I.H., Son, H.Y., Cho, S.W., Ha, C.S. and Kang, B.H. (2003) Zearalenone induces male germ cell apoptosis in rats. Toxicol. Lett. 138, 185–192.
[11] Ouanes, Z., Abid, S., Ayed, I., Anane, R., Mobio, T., Creppy, E.E. and Bacha, H. (2003) Induction of micronuclei by zearalenone in Vero monkey kidney cells and in bone marrow cells of mice: protective effect of vitamin E. Mutat. Res. 538, 63–70.
[12] IARC (1993) Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins in: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, vol. 56 (IARC, Ed.), pp. 397–444, IARC, Lyon, France.
[13] Pfohl-Leszkowicz, A., Chekir-Ghedira, L. and Bacha, H. (1995) Genotoxicity of zearalenone, an oestrogenic mycotoxin: DNA adducts formation in female mouse tissues. Carcinogenesis 16, 2315–2320.
[14] Abid-Essefi, S., Ouanes, Z., Hassen, W., Baudrimont, I., Creppy, E. and Bacha, H. (2004) Cytotoxicity, inhibition of DNA and protein syntheses and oxidative damage in cultured cells exposed to zearalenone. Toxicol. In Vitro 18, 467–474.
[15] Yu, Z., Zhang, L., Wu, D. and Liu, F. (2005) Anti-apoptotic action of zearalenone in MCF-7 cells. Ecotoxicol. Environ. Saf. 62, 441–446.
[16] Moggs, J.G., Ashby, J., Tinwell, H., Lim, F.L., Moore, D.J., Kimber, I. and Orphanides, G. (2004) The need to decide if all estrogens are intrinsically similar. Environ. Health Perspect. 112, 1137–1142.
[17] Buterin, T., Koch, C. and Naegeli, H. (2006) Convergent transcriptional profiles induced by endogenous estrogen and distinct xenoestrogens in breast cancer cells. Carcinogenesis 27, 1567–1578.
[18] Dip, R., Lenz, S., Antignac, J.P., Le Bizec, B., Gmuender, H. and Naegeli, H. (2008) Global gene expression profiles induced by phytoestrogens in human breast cancer cells. Endocr. Relat. Cancer 15, 161–173.
[19] Inoue, T. (2003) Toxicogenomics – a new paradigm of toxicology in: Toxicogenomics (Inoue, T. and Pennie, W.D., Eds.), Springer-Verlag, Tokyo, Japan. pp. 213–218.
[20] Terasaka, S., Aita, Y., Inoue, A., Hayashi, S., Nishigaki, M., Aoyagi, K., Sasaki, H., Wada-Kiyama, Y., Sakuma, Y., Akaba, S., Tanaka, J., Sone, H., Yonemoto, J., Tanji, M. and Kiyama, R. (2004) Expression profiling of the estrogen responsive genes for evaluation of estrogen activity among natural estrogens and industrial chemicals using a customized DNA microarray. Environ. Health Persp. 112, 773–781.
[21] Ise, R., Han, D., Takahashi, Y., Terasaka, S., Inoue, A., Tanji, M. and Kiyama, R. (2005) Expression profiling of the estrogen responsive genes in response to phytoestrogens using a customized DNA microarray. FEBS Lett. 579, 1732–1740.
[22] Terasaka, S., Inoue, A., Tanji, M. and Kiyama, R. (2006) Expression profiling of estrogen-responsive genes in breast cancer cells treated with alkylphenols, chlorinated phenols, parabens, or bis- and benzoylphenols for evaluation of estrogenic activity. Toxicol. Lett. 163, 130–141.
[23] Dong, S., Inoue, A., Zhu, Y., Tanji, M. and Kiyama, R. (2007) Activation of rapid signaling pathways and the subsequent transcriptional regulation for the proliferation of breast cancer MCF-7 cells by the treatment with an extract of glycyrrhiza glabra root. Food Chem. Toxicol. 45, 2470–2478.
[24] Parveen, M., Inoue, A., Ise, R., Tanji, M. and Kiyama, R. (2008) Evaluation of estrogenic activity of phthalate esters by gene expression profiling using a focused microarray (EstrArray). Environ. Toxicol. Chem. 27, 1416–1425.
[25] Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S. and Boyd, M.R. (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82, 1107–1112.
[26] Eisen, M.B., Spellman, P.T., Brown, P.O. and Botstein, D. (1998) Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. USA 1998 (95), 14863–14868.
[27] Edgar, R., Domrachev, M. and Lash, A.E. (2002) Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 30, 207–210.
[28] Greco, S., Storelli, C. and Marsigliante, S. (2006) Protein kinase C (PKC)-delta/-epsilon mediate the PKC/Akt-dependent phosphorylation of extracellular signal-regulated kinases 1 and 2 in MCF-7 cells stimulated by bradykinin. J. Endocrinol. 188, 79–89.
[29] Shier, W.T., Shier, A.C., Xie, W. and Mirocha, C.J. (2001) Structure-activity relationships for human estrogenic activity in zearalenone mycotoxins. Toxicon 39, 1435–1438.
[30] Minervini, F., Giannoccaro, A., Cavallini, A. and Visconti, A. (2005) Investigation on cellular proliferation induced by zearalenone and its derivatives in relation to the estrogenic parameters. Toxicol. Lett. 159, 272–283.
[31] Butt, A.J., Dickson, K.A., McDougall, F. and Baxter, R.C. (2003) Insulin-like growth factor-binding protein-5 inhibits the growth of human breast cancer cells in vitro and in vivo. J. Biol. Chem. 278, 29676–29685.
[32] Liu, H., Du, J., Hu, C., Qi, H., Wang, X., Wang, S., Liu, Q. and Li, Z. (2009) Delayed activation of extracellular-signal-regulated kinase 1/2 is involved in genistein- and equol-induced cell proliferation and estrogen-receptor-alpha-mediated transcription in MCF-7 breast cancer cells. J. Nutr. Biochem. (in press).
[33] Lau, W.S., Chen, W.F., Chan, R.Y., Guo, D.A. and Wong, M.S. (2009) Mitogen-activated protein kinase (MAPK) pathway mediates the oestrogen-like activities of ginsenoside Rg1 in human breast cancer (MCF-7) cells. Br. J. Pharmacol. 156, 1136–1146.
[34] Razandi, M., Pedram, A., Merchenthaler, I., Greene, G.L. and Levin, E.R. (2004) Plasma membrane estrogen receptors exist and functions as dimers. Mol. Endocrinol. 18, 2854–2865.
[35] Pedram, A., Razandi, M. and Levin, E.R. (2006) Nature of functional estrogen receptors at the plasma membrane. Mol. Endocrinol. 20, 1996–2009.
[36] Revankar, C.M., Cimino, D.F., Sklar, L.A., Arterburn, J.B. and Prossnitz, E.R. (2005) A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science 307, 1625–1630.
[37] Thomas, P., Pang, Y., Filardo, E.J. and Dong, J. (2005) Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. Endocrinology 146, 624–632.