Tissue-specific estrogenic and non-estrogenic effects of a xenoestrogen, nonylphenol

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Abstract

Alkylphenols perturb the endocrine system and are considered to have weak estrogenic activities. Although it is known that nonylphenol can bind weakly to the estrogen receptor, it is unclear whether all reported effects of nonylphenol are attributable to its estrogen receptor-binding activity. In order to examine whether alkylphenols have similar effects to the natural hormone, estradiol, we used a mouse model to examine the effects of nonylphenol on gene expression and compared it with estradiol. DNA microarray analysis revealed that, in the uterus, most of the genes activated by this alkylphenol at a high dose (50 mg/kg) were also activated by estradiol. At lower doses, nonylphenol (0.5 mg/kg and 5 mg/kg) had little effect on the genes that were activated by estradiol. Thus, we concluded that the effects of nonylphenol at a high dose (50 mg/kg) were very similar to estradiol in uterine tissue. Moreover, since evaluation of estrogenic activity by gene expression levels was comparable with the uterotrophic assay, it indicated that analysis of gene expression profiles can predict the estrogenic activities of chemicals. In contrast to the similar effects of nonylphenol and estradiol observed in the uterus, in the liver, gene expression was more markedly affected by nonylphenol than by estradiol. This indicated that, in the liver, nonylphenol could activate another set of genes that are distinct from estrogen-responsive genes. These results indicated that nonylphenol has very similar effects to estradiol on gene expression in uterine but not in liver tissue, indicating that tissue-specific effects should be considered in order to elucidate the distinct effects of alkylphenols.

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Introduction

There has recently been considerable concern about the potential endocrine-disrupting effects of chemicals released into the environment. Those chemicals that have estrogenic activity are termed xenoestrogens or endocrine disruptors. They are thought to mimic or disturb the function of estrogen and many are known to possess estrogen receptor-binding activity (Danzo 1997). Among the xenoestrogens, alkylphenols have been recognized to bind to estrogen receptors and exert weak estrogenic activities *in vivo* and *in vitro* (Mueller & Kim 1978, White *et al.* 1994). The estrogenic activity of alkylphenol compounds has been reported not only in laboratory-based studies (Soto

et al. 1991, Shelby *et al.* 1996), but also in wildlife (Tyler *et al.* 1998), especially fish (Flouriot *et al.* 1995, Sumpter & Jobling 1995).

Since the original discovery of the estrogenic activity of alkylphenols, many further studies have reported diverse adverse effects of these compounds. For example, in animals exposed to nonylphenol, the weight of the epididymis was decreased (Hossaini *et al.* 2001), sperm function was affected (Chapin *et al.* 1999, Adeoya-Osiguwa *et al.* 2003) and deleterious effects on mammary gland and uterine function were observed (Odum *et al.* 1999, Danzo *et al.* 2002).

Despite these studies, the precise mechanisms underlying the adverse effects of endocrine-disrupting chemicals are poorly understood.

Although it is well established that nonylphenol binds the estrogen receptor, it is not certain that all effects of nonylphenol can be explained by this binding activity. To determine whether the diverse effects of nonylphenol are wholly attributable to its estrogen receptor-binding activity, it is essential to compare nonylphenol and estradiol with respect to gene activation. Thus, we here analyzed gene expression patterns caused by nonylphenol and estradiol using microarray technology.

Materials and methods

Animals

Animals were housed under a 12 h light:12 h darkness cycle. ER α knockout (α ERKO) mice (Lubahn *et al.* 1993) and their wild-type counterparts (C57/BL6/J background) were ovariectomized at 8 weeks of age. After 2 weeks, the ovariectomized mice were injected intraperitoneally with a chemical or sesame oil (Nakarai Tesque, Kyoto, Japan) as a vehicle control and whole uteri ($n=4$) were collected 6 h after dosage. Doses of estrogens used were: 50 μ g/kg body weight (BW); 5 μ g/kg BW; 0.5 μ g/kg BW; and 0.05 μ g/kg BW for 17 β -estradiol (Sigma, Sigma-Aldrich Japan, Tokyo, Japan) and 50 mg/kg BW; 5 mg/kg BW; and 0.5 mg/kg BW for nonylphenol (Nakarai Tesque). For the uterotrophic assay, ovariectomized mice were injected intraperitoneally with estrogen or sesame oil every 24 h for 7 days and whole uteri ($n=5$) were collected and weighed 6 h after the last dosage. The tissues were then fixed in formalin, embedded in paraffin, cut into 8 μ m sections and the number of glands was counted. All animal experiments were approved by the institutional Animal Care Committee.

DNA microarray analysis

Total uterine RNA was extracted using TRIZOL (Invitrogen, Tokyo, Japan) and purified using an RNeasy mini kit (Qiagen, Tokyo, Japan). Purified RNA was labeled with biotin according to the manufacturer's protocol and hybridized with a mouse genome U74Av2 array (Affymetrix Japan, Tokyo, Japan). After washing, the array was scanned to measure fluorescent intensity that is representative of gene expression.

The fluorescent intensity of each probe was further analyzed by dChip, a model-based expression analysis program (Baccarelli *et al.* 2002) and expression levels were estimated. For the analysis, a perfect match (PM)-only model (Baccarelli *et al.* 2002) was used. The estimated values (gene expression levels) were transferred to the GeneSpring software program (Silicon Genetics, Redwood City, CA, USA) and analyzed. To deduce credible gene expression levels from DNA microarray analysis, we independently repeated each experiment at least twice, with averaged values being used for the analysis.

For the clustering analysis, genes whose expression levels exceeded 1000 were selected and similarities between experiments and genes were measured by standard correlation using the GeneSpring program. After selecting the genes that were activated more than twofold by nonylphenol, k-means analysis was performed using the GeneSpring program beginning from five random starting clusters.

Quantitative real-time PCR

Total RNA was purified as described above. cDNA was synthesized from purified total RNA by Superscript II RT(–) (Invitrogen) with random primers at 42 °C for 60 min. PCR reactions were performed in the PE Prism 5700 sequence detector (PE Biosystems, Tokyo, Japan) using SYBR-Green PCR core reagents (PE Biosystems) in the presence of appropriate primers according to the manufacturer's instructions. Each PCR amplification was performed in triplicate under the following conditions: 2 min at 50 °C and 10 min at 95 °C, followed by a total of 40 two temperature cycles (15 s at 95 °C and 1 min at 60 °C).

Gene expression levels were normalized to the expression levels of L8 mRNA (U67771) and gel electrophoresis and melting curve analyses were performed to confirm correct amplicon size and the absence of non-specific bands.

The primers were chosen to amplify short PCR products of less than 100 base pairs and their sequences are as follows. X79003 (integrin α 5), CCCGAAGAGCCAGTCCAAT, CCCAGCCC TCAGCACCTT; L32752 (Ran GTPase), TTG GAGTTCGTTGCCATGC, ACTGTGCTGCCA AAGCTGG; L40406 (hsp-E7I), GGAACGACC GAAAGTGTG, CCTCTGAAGTCCGCTG

Table 1 Correlation coefficients between repetitive experiments for the same condition

Oil	Exp 1	Exp 2	Exp 3	Exp 4
Exp 1	1.00	0.94	0.97	0.93
Exp 2		1.00	0.92	0.94
Exp 3			1.00	0.90
Exp 4				1.00
E2 (5 µg)	Exp 1	Exp 2	Exp 3	
Exp 1	1.00	0.91	0.92	
Exp 2		1.00	0.96	
Exp 3			1.00	
NP (0.05 mg)	NP (0.5 mg)	NP (50 mg)		
0.96	0.95	0.93		
E2 (0.05 µg)	E2 (0.5 µg)	E2 (50 µg)		
0.96	0.96	0.96		

Control experiments (oil) were repeated four times and correlation coefficients between each experiment are indicated in a matrix. Estradiol treatments (5 µg/kg BW) were independently repeated three times and the correlation coefficient between each experiment is indicated as a matrix. Other experiments were repeated twice for each condition and the correlation coefficients between two experiments are indicated for the compounds and doses in the non-matrix section. E2, estradiol; NP, nonylphenol. The doses (per kg BW) are indicated in parentheses.

CAAT; U90446 (RNAse L inhibitor), GAGCAAC CAAGGACAACCTAAAAC, TGCTATTTCCC TGCCTCACTG; Z18272 (collagen $\alpha 2$ type VI), CCATTGCCTGTGACAAGCC, CTCGGACA CCAGGTCAGAGAA; AF000236 (orphan chemokine receptor), AGGACCGGATGTCTCTCC ATT, GTTGATGAGCTCTGTTGATTGACG; U67771 (ribosome L8) ACAGAGCCGTTGTTGG TGTTG, CAGCAGTTCCTCTTTGCCTTGT.

Results

Reproducibility of the DNA microarray analysis

In order to compare the changes in gene expression caused by nonylphenol and estradiol, we administered either agent to mice and examined gene expression profiles by DNA microarray analysis. The reproducibility of the experiments was evaluated by calculating correlation coefficients between experiments under the same conditions as shown in Table 1. As all combinations of experiments for the same condition had a

correlation coefficient of at least 0.9, our DNA microarray data was highly reproducible.

Dose-dependent gene activation by estradiol and nonylphenol

In response to increasing amounts of nonylphenol or estradiol, gene expression levels changed significantly (Fig. 1). The r^2 value calculated between treated and control gene expression levels changed dose dependently with increasing doses of estradiol (Table 2), but that of nonylphenol was lower at 0.5 than at 5 mg/kg. In agreement with these r^2 values, gene expression was modulated more significantly at 0.5 mg/kg nonylphenol than at 5 mg/kg nonylphenol. As the number of genes activated by nonylphenol at the highest dose (50 mg/kg) (307 genes) was greater than for estradiol (238 genes; Table 2), we examined its influence on the gene expression pattern in more detail.

Evaluation of relative estrogenic activity of nonylphenol from gene expression patterns

To examine whether the genes activated by nonylphenol were similar to those activated by estradiol, we selected 307 genes that were induced more than twofold at the highest dose of nonylphenol (Table 2) and performed hierarchical clustering analysis. As shown in the dendrogram (Fig. 2), the expression pattern caused by the highest dose of nonylphenol was equivalent to that observed between 5 and 0.5 µg/kg estradiol. Although one cluster of genes had a dose-dependent gene activation pattern (Fig. 2), the other cluster showed no prominent dose-dependent gene activation.

Genes activated by nonylphenol are also activated by estradiol

As clustering analysis indicated that the gene activation patterns of nonylphenol and estradiol were similar, we further examined whether the genes activated by nonylphenol were the same as those activated by estradiol. We applied the k-means method to the selected 307 genes to determine if they were also activated by estradiol. If some genes were activated only by nonylphenol, the presence of a cluster that was activated by

Figure 1 Scatter plot of gene expression levels changed by nonylphenol or estradiol in the uterus. Ovariectomized mice were given either nonylphenol or estradiol and gene expression levels were evaluated by DNA microarray analysis after 6 h. The gene expression levels (see Materials and methods) of treated and control animals were compared by scatter plot. The X axis indicates gene expression levels in control (oil-treated) and the Y axis indicates the gene expression levels in treated mice. Each treatment is indicated in the respective panel with its dose. NP, nonylphenol; E2, estradiol.

Table 2 Number of genes whose expression levels were activated or repressed more than twofold

	Estradiol (µg/kg)				Nonylphenol (mg/kg)		
	0.05	0.5	5	50	0.5	5	50
FC >2	0	14	218	238	38	1	307
FC <0.5	5	2	28	61	29	21	162
r ²	0.99	0.98	0.95	0.94	0.97	0.98	0.91

Number of genes that were activated more than twofold (FC >2) or repressed more than twofold (FC <0.5) are indicated. Equations of regression lines were calculated from scatter plots in Fig. 1 (treated vs control) and *r*² values were calculated.

nonylphenol, but not by estradiol, could be expected. However, even when we increased the number of clusters, we could not identify any cluster that clearly indicated the presence of genes activated by nonylphenol but not estradiol. Figure 3 shows the result of clustering using three clusters. Among the three gene clusters, two clusters (Fig. 3B–C) showed a rather similar pattern. They were activated by estradiol in a dose-dependent manner and activated by nonylphenol only at the highest dose but exhibited no clear dose-dependent pattern in response to nonylphenol. One cluster (Fig. 3A) showed an atypical pattern compared with the other clusters, with those genes being activated

more markedly at the highest dose of nonylphenol. Importantly, dose-dependent gene activation by estradiol correlated with uterotrophic effects, as shown in Fig. 3D. These results indicated that estrogenic activities of nonylphenol can also be estimated by gene expression profiling. The total number of genes activated more than twofold by nonylphenol (307 genes) was greater than that for estradiol (238 genes). These genes were mainly contained within one cluster (Fig. 3A and data not shown), but they were not nonylphenol specific because they were also activated by estradiol, albeit with weaker effects. As shown in Fig. 3, almost all genes that were activated by the high dose of

Figure 2 Clustering analysis of nonylphenol-induced genes. For the analysis, 307 genes activated by nonylphenol more than twofold at a dose of 50 mg/kg were selected and expression values under each condition were calculated. The calculated expression levels were then clustered. Treatments are indicated for the respective lanes. A cluster of genes that is representative of the dendrogram is indicated by a solid bar on the right. A cluster of genes that does not correlate with the dose is indicated by an open bar. Numbers indicate the dose administered (mg/kg). NP, nonylphenol; E2, estradiol.

nonylphenol or by estradiol were not activated in ERKO mice. Thus, those genes that were upregulated by both nonylphenol and estradiol were activated by the estrogen receptor, either directly or indirectly. These similar gene activation patterns were confirmed by quantitative real-time PCR (Fig. 4).

Gene activation profile of nonylphenol in liver

As our results in the uterus suggested that nonylphenol and estradiol have similar effects on gene expression, at least at some doses, we examined whether the effects of these chemicals were also similar in another tissue, liver, again using DNA microarrays. As 5 µg/kg estradiol and 50 mg/kg nonylphenol could activate gene expression to similar extents in the uterus (Fig. 5A and C), we administered these doses and examined resultant changes in liver gene expression. This indicated that in liver, nonylphenol activated more genes than estradiol (Fig. 5B and C). Calculation

of fold change of each gene by estradiol or nonylphenol indicated that in liver, gene activation induced by nonylphenol was different from that induced by estradiol (Fig. 5C). The list of the genes that were activated more markedly by nonylphenol than by estradiol is shown in Table 3. Overall, our results indicated that nonylphenol and estradiol similarly affect uterine gene expression but have different effects on liver gene expression.

Discussion

In this study, we examined whether nonylphenol has effects on gene expression that are distinct from estrogenic effects, by comparing its effects on gene expression with those of estradiol. Several doses were used since comparison of changes in gene expression at one dose cannot be used to validly estimate the activities of chemicals (Watanabe *et al.* 2003). In summary, we demonstrated that (1) the estrogenic activity of nonylphenol could be

Figure 3 k-means analysis of nonylphenol-activated genes. To examine dose-dependent gene activation patterns, genes that were activated by nonylphenol more than twofold were selected and clustered by k-means (number of class was three). Average values of fold changes in each cluster are indicated in each panel (A–C). Numbers of the clustered genes are indicated at the top right of each panel. KO indicates the gene activation profile of ERKO mice. Oil, control; E2, 5 µg/kg estradiol; NP, 50 mg/kg nonylphenol. The dose ranges were 0.05, 0.5, 5 and 50 µg/kg for estradiol and 0.5, 5 and 50 mg/kg for nonylphenol, which are indicated on the X axis. (D) The results of the uterotrophic assay (mg of uterine weight/g BW) are indicated by the solid line and the numbers of glands under the same conditions are indicated by the broken line.

estimated from gene expression profiling, and this was comparable with results obtained in the uterotrophic assay; (2) almost all genes activated by estradiol in the uterus were also activated by nonylphenol at a high dose; and (3) nonylphenol and estradiol have similar effects on gene expression in the uterus, but not in the liver.

Our clustering analysis indicated that the relative activity of nonylphenol estimated from gene expression levels was between 0.001 and 0.0001 (relative to an activity of estradiol equal to 100)

(Fig. 2), which was consistent with the value of 0.00036 estimated in a previous study (Coldham *et al.* 1997). This value was also consistent with previous estimations using the uterotrophic assay and other studies using other systems such as E-screen (Soto *et al.* 1995, Kwack *et al.* 2002), a yeast reporter system (Coldham *et al.* 1997, Andersen *et al.* 1999) and a ligand-binding assay (Coldham *et al.* 1997, Andersen *et al.* 1999).

Our analysis also found that lower doses of nonylphenol did not exhibit estrogenic effects on

Figure 4 Quantification of mRNA by real-time PCR. Selected gene expression levels, as identified by DNA microarray analysis, were examined by real-time PCR. Nonylphenol (NP) or estradiol (E2) treatments were repeated three times and evaluations of gene expression changes were performed independently. Average values of the fold changes and their error are indicated. GeneBank accession numbers of the selected genes are indicated. X79003, integrin $\alpha 5$; L32752, GTPase (Ran); L40406, hsp-E7I; U90446, RNase L inhibitor; Z18272, collagen $\alpha 2$ chain type VI; AF000236, orphan chemokine receptor.

gene expression. Although genes were activated in a dose-dependent manner by estradiol in three orders of magnitude (0.5–50 $\mu\text{g/kg}$), they were activated by only one dose (50 mg/kg) of nonylphenol and there was no clear dose–response by nonylphenol. This is clearly illustrated in Fig. 2

where a mid-range dose of nonylphenol (5 mg/kg) is not positioned between 5 $\mu\text{g/kg}$ and 0.5 $\mu\text{g/kg}$ estradiol. Although k-means analysis indicated subsets of genes that were activated at 0.5 mg/kg nonylphenol, this activation pattern was distinct from the effects of estradiol, as indicated in Fig. 3. This indicated that the effect of nonylphenol at lower doses is not due to the general estrogenic activity of the chemical and that nonylphenol has estrogenic effects only at the highest dose.

In addition to the above findings, another important finding in this study was that the difference in gene activation profiles between nonylphenol and estradiol was much greater in the liver than in the uterus. Nonylphenol-specific changes in gene expression were not prominent in the uterus, whereas in the liver the number of nonylphenol-activated genes was very different from that of estradiol, indicating that their effects on gene expression are tissue specific. This indicated that evaluations of chemical activities which rely on one cell line may be insufficient for validation, and the use of tissues may be critical in order to fully understand the chemical effects.

With regard to the genes that were activated by nonylphenol but not by estradiol in the liver, many genes involved in lipid or fatty acid metabolism were detected in addition to cytochrome P450 genes. These included apolipoprotein A-IV, peroxiredoxin, granulin and lecithin cholesterol acyltransferase, which are involved in lipid metabolism, and acetyl-CoA acyltransferase, which is involved in the metabolism of fatty acids. In rodents, it has been

Figure 5 Scatter plots and fold changes of gene expression levels in the liver and uterus. Gene expression levels in nonylphenol (NP)- or estradiol (E2)-treated uteri (Y axis) were plotted against control gene expression levels (X axis) (A) and those in the liver were also plotted (B). (C) Fold changes (ratio of treated gene expression level to control gene expression level) were calculated and plotted on a log scale ($\log_2$).

Table 3 List of genes specifically activated by nonylphenol but not by estradiol in the liver

GeneBank number	log2		Title (annotations, June 2003)
	NP/oil	E2/oil	
M24264	3.86	1.37	Cytochrome P450, family 2, subfamily d, polypeptide 10
AB025217	3.27	0.81	Calcium-binding protein P22
M76131	3.07	0.89	Eukaryotic translation elongation factor 2
AF093853	2.82	1.05	Peroxiredoxin 6
AB003304	2.69	1.04	Proteasome (prosome, macropain) subunit, β type 5
D87898	2.68	0.94	ADP-ribosylation factor 1
V00714	2.60	0.26	Hemoglobin α , adult chain 1
AB020203	2.59	0.16	Adenylate kinase 3 α -like
U03715	2.55	0.58	Procollagen, type XVIII, α 1
AW045202	2.47	0.63	Protein disulfide isomerase-related protein
L39879	2.44	0.78	Ferritin light chain 1
D17571	2.40	0.73	P450 (cytochrome) oxidoreductase
M27134	2.38	0.26	Histocompatibility 2, Q region locus 10
AI839702	2.35	0.51	Copper chaperone for superoxide dismutase
Z97207	2.34	0.69	Butyrate-induced transcript 1
AF115480	2.28	0.67	cAMP-regulated guanine nucleotide exchange factor II
X57024	2.27	0.55	Glutamate dehydrogenase
AV003378	2.26	0.05	Hemoglobin α , adult chain 1
M64248	2.24	0.65	Apolipoprotein A-IV
AW012807	2.23	0.87	GrpE-like 1, mitochondrial
AI530403	2.21	0.43	Acetyl-coenzyme A acyltransferase 1
L47335	2.20	0.43	Branched chain ketoacid dehydrogenase E1, α polypeptide
U65403	2.20	0.92	Seum amyloid A 4
L12140	2.20	0.44	Amino-terminal enhancer of split
U35741	2.20	0.42	Thiosulfate sulfotransferase, mitochondrial
AI842889	2.17	0.21	ATPase, H ⁺ transporting V0 subunit B
AF030065	2.16	0.43	Hepsin
J05154	2.16	0.46	Lecithin cholesterol acyltransferase
AW045202	2.15	0.43	Protein disulfide isomerase-related protein
X17069	2.15	0.44	FK506 binding protein 4
AA795613	2.15	0.50	Transcription elongation factor B (SIII), polypeptide 2 (18 kDa, elongin B)
X76850	2.13	0.92	MAP kinase-activated protein kinase 2
D87896	2.12	0.56	Glutathione peroxidase 4
X66091	2.12	0.91	Splicing factor, arginine/serine-rich 1 (ASF/SF2)
AF091390	2.09	0.43	FXD domain-containing ion transport regulator 1
J00413	2.09	0.71	Hemoglobin, β adult major chain
AI853523	2.09	1.06	Ubiquinol-cytochrome c reductase subunit
AW045751	2.07	-0.36	Quiescin Q6
AW122731	2.05	0.20	Diaphorase 1 (NADH)
X05021	2.05	0.58	Ribosomal protein L27a
AW123952	2.05	0.70	ATPase, Na ⁺ /K ⁺ transporting, α 1 polypeptide
D16333	2.05	0.19	Coproporphyrinogen oxidase
J05185	2.04	0.64	Prolyl 4-hydroxylase, β polypeptide
AW123556	2.04	0.42	Quiescin Q6
AW120711	2.04	0.32	DNAJ (Hsp40) homolog, subfamily B, member 9
X04435	2.03	-0.08	Nuclear receptor subfamily 3, group C, member 1
U29402	2.03	0.46	Ribosomal protein, large, P1
M77196	2.02	-0.08	CEA-related cell adhesion molecule 1
L00919	2.02	0.32	Erythrocyte protein band 4.1
AA838868	2.01	0.10	Latent transforming growth factor β binding protein 4
L31777	2.01	0.45	Triosephosphate isomerase
D16195	2.00	0.36	Granulin

The genes with GeneBank accession numbers that were activated more than fourfold by nonylphenol are indicated with fold changes. Fold changes (log2) calculated from the ratio of gene expression levels of nonylphenol (NP) to those of the control (oil) are indicated in the second column. Fold changes (log2) calculated from the ratio of gene expression levels of estradiol (E2) to those of the control (oil) are indicated in the third column.

reported that estrogen does not affect the expression of lecithin cholesterol acyltransferase (Staels *et al.* 1992) and that it downregulates the expression of apolipoprotein A-IV (Staels *et al.* 1990) in the liver – observations with which our results are consistent. Thus activation of these genes in the liver by nonylphenol may not be an estrogenic affect of nonylphenol.

In rodents, peroxisome proliferators are known to affect the expression of genes involved in lipid metabolism. Although peroxisome proliferator-activated receptors (PPARs) are candidate nonylphenol-responsive transcription factors, a genome-wide gene expression pattern of PPAR α agonists did not overlap with our data, even though the same system was used for the analysis (Yamazaki *et al.* 2002).

In the liver, steroid and xenobiotic receptor (SXR)/pregnenolone X receptor (Blumberg *et al.* 1998, Moore *et al.* 2000) is also known to respond to xenobiotics and to activate a subset of genes (Masuyama *et al.* 2000, Xie *et al.* 2000), but it was not clear whether the genes specifically activated by nonylphenol in liver are driven by SXR, since very few SXR-responsive genes (Rosenfeld *et al.* 2003) are activated by nonylphenol. For example, although CYP3A is one of the prominent genes activated by SXR, here the CYP genes that were activated were CYP2d10, CYP oxidoreductase and ubiquinol-cytochrome c reductase. It is possible that in the liver, other transcription factors, such as hepatocyte nuclear factors, may activate genes more strongly than the estrogen receptor. Thus, further study is necessary to clarify the molecular mechanisms of the tissue-specific effects of nonylphenol.

Taken together, our analysis of gene expression not only elucidated the identity of responsive genes and differences in their sensitivities to nonylphenol and estradiol, but also shed light on the tissue-specific effects. These findings will be important for understanding the diverse effects of xenoestrogens.

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