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Heterologous Microarray Hybridization Used for Differential Gene Expression Profiling in Benzo[a]pyrene-exposed Marine Medaka

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Accepted 9 October 2009

Abstract

Differential gene expression profiling was performed in the hepatic tissue of marine medaka fish (*Oryzias javanicus*) after exposure to benzo[a]pyrene (BaP), a polycyclic aromatic hydrocarbon (PAH), by heterologous hybridization using a medaka cDNA microarray. Thirty-eight differentially expressed candidate genes, of which 23 were induced and 15 repressed ($P < 0.01$), were identified and found to be associated with cell cycle, development, endocrine/reproduction, immune, metabolism, nucleic acid/protein binding, signal transduction, or non-categorized. The presumptive physiological changes induced by BaP exposure were identified after considering the biological function of each gene candidate. The results obtained in this study will allow future studies to assess the molecular mechanisms of BaP toxicity and the development of a systems biology approach to the stress biology of organic chemicals.

Keywords: Marine medaka, *Oryzias javanicus*, Heterologous hybridization, Microarray benzo[a]pyrene (BaP), Differential gene expression profile

The genome-wide analysis of gene expression has recently become viable due to the development of cDNA and oligonucleotide microarrays. Microarray technology is considered a powerful tool and is able to be applied to various fields of biological science, such as developmental biology, physiology and toxicology. Further, it has been employed in differential gene expression profiling, microbial detection, SNPs genotyping and comparative genome hybridization. However, microarrays are restricted to only a few traditional model species as well as human, as the genome structure of these organisms has been elucidated and extensive gene expression data have been accumulated. For non-model organisms that lack sufficient genome-wide gene expression data, microarray technology cannot be applied. To overcome this limitation, heterologous or cross-species microarray hybridization analyses[1] have been performed in a variety of non-model species for the monitoring of differential gene expression to changed physiological conditions[2,3], and/or for the comparison of gene expression between two independent species[4]. Among these approaches, the ecotoxicological applications capable of identifying environmentally responsive genes are particularly important, as almost all species living in nature are not traditional model organisms[5].

Polycyclic aromatic hydrocarbons (PAHs) are the most persistent organic pollutants among all worldwide aquatic environments. PAH contamination rises every year due to such anthropogenic activities as incomplete burning of fossil fuels and organic substances to oil spill accidents. Its bioaccumulative potential has been reported extensively in many organisms, including aquatic plant[6], mussels[7], polychaetes worm[8], and fishes[9]. Among PAHs, Benzo[a]pyrene (BaP; CASRN: 50-32-8), a representative ecotoxicant, has been extensively characterized to produce carcinogenetic effects and immune function suppression[10] in test animals. The toxic effects of BaP during the early life stage of an organism inhibit development to adult, which could conceivably cause disturbances in the food chain of an ecosystem as well as serious economic loss in fishing industries. Therefore, it is necessary to detect the biological effects of a pollutant on biological organisms even at levels under contamination as well as

the effects on the health status of the ecosystem.

The importance of fish models in environmental genomic studies has been well-documented[11]. However, at present there are only a few marine fishes, such as Sheepshead minnow, Atlantic silverside and Tidewater silverside, that have been recommended as toxicological test fishes by international organizations[12]. This low number of marine test fishes is due to the abundance and convenience of freshwater test fishes recommended so far. Thus, there is a strong need for the development of new marine test fishes. Javanese medaka (*Oryzias javanicus*) is a good candidate for this purpose, as their sensitivity to chemicals at the environmentally relevant concentrations has already demonstrated by several research groups[13-15]. In the present study, a cDNA microarray which constructed from a Japanese medaka fish (*Oryzias latipes*) [Medaka 750 and Medaka Early-stage Embryo 2200 array (Ecogenomics Inc., Fukuoka, Japan)] was introduced in order to generate a differential gene expression profile of the hepatic tissue of marine medaka fish (*Oryzias javanicus*) upon exposure to BaP. This study provides an initial identification of BaP responsive genes that can be used as a basis for future investigations into the potential biomarkers of BaP exposure, the toxic mechanism of BaP, and finally the BaP-mediated molecular mechanism.

Differentially Expressed Genes in BaP-Exposed Marine Medaka

To identify the genes associated with BaP-induced toxicity, gene expression profiling in the liver of BaP-exposed marine medaka (100 ppb, 24 h) was performed using a Japanese medaka cDNA microarray containing about 3,055 medaka genes. From the cDNA microarray analysis, 38 reliable genes were found to have transcript levels affected by BaP exposure ($P < 0.01$). Of these genes, the expression of 23 was induced, whereas that of 15 was repressed (Table 1). The 38 differentially expressed genes could be categorized into 8 groups: cell cycle (2, 5.3%), development (4, 10.5%), endocrine/reproduction, (9, 23.7%), immune (4, 10.5%), metabolism (9, 23.7%), nucleic acid/protein binding (7, 18.4%), signal transduction (2, 5.3%), and non-categorized (1, 2.6%).

Discussion

One goal of this study was to estimate the potential effect of BaP on the new marine test fish marine medaka (*O. javanicus*) using a heterologous microarray hybridization. The initial screening of liver tissue of marine medaka after exposure to BaP produced 38 differentially expressed genes that could be involved in carcinogenesis, endocrine disruption, circulatory problems, immune responses, detoxification, molecular chaperones, response to hypoxia, neuronal deficiencies and other biological responses. Possible interpretations of these gene expression profiles are discussed below, although it remains difficult to draw conclusions.

Carcinogenesis

D-type cyclins (Cyclin D1, D2, and D3) serve as initial controllers of the cell cycle. Overexpression of cyclin D2 has shown to shorten the G0- to S-phase interval in rodent fibroblasts[16] and is known to promote the invasiveness of human squamous carcinoma cells[17]. In our microarray analysis, the mRNA expression level of cyclin D2 was approximately 2.90-fold higher in the group exposed to BaP (Table 1).

Receptor of activated protein kinase C1 (RACK1) is a member of the protein tyrosine phosphatase (PTP) family. These signaling molecules have functions in regulating cellular processes including cell growth, adhesion, differentiation, mitotic cycle and oncogenic properties. Recently, increased RACK1 expression was suggested as an important predictor for a specific type of carcinoma[18]. There was a significant increase in RACK1 transcript level to 2.29-fold in the BaP-exposed group (Table 1).

Growth differentiation factor 11 (Gdf11) is known to a play role in the regulation of development and differentiation. Recently, the transcript level of Gdf11 was shown to be significantly higher in specimens obtained from colorectal cancer patients[19]. Our microarray experiment showed that the mRNA level of Gdf-11 was increased 2.90-fold in the BaP-exposed group (Table 1), suggesting BaP might induce a certain type of cancer in marine medaka.

Annexins are a gene family of calcium- and phospholipid-binding proteins that play key roles in regulating a variety of signal transduction mechanisms, including channel formation, membrane fusion, vesicle transport and phospholipase A_2 activity. Recent studies suggest that differentially expressed annexin genes are related to specific types of tumor development[20]. Among various types of annexins, the annexin max4 gene, a medaka homologue of human annexin11 (Anxa11), was found to be overexpressed in liver tissue of BaP-exposed marine medaka (2.50-fold) (Table 1).

Histone acetylation plays an important role in the regulation of eukaryotic gene expression. The histone deacetylase (HDAC) complex interacts with retinoblastoma tumor-suppressor protein to form a complex that is key in the control of cell proliferation and differentiation. The expression of a specific subset of genes (7%) involved in growth control and cell communica-

Table 1. Induction and repression of gene expression after BaP exposure in *O. javanicus* was obtained by heterologous microarray analysis. The numbers indicate significant differences in fold induction or repression relative to expression in the control group ($P < 0.01$).

Category	Gene	FD*	Accession No.
	Up-regulated		
Cell cycle	Cyclin D2	2.90	AU241108
	Receptor of activated protein kinase C1 (RACK1)	2.29	AF025331.1
Development	HOXC5A	3.86	AB026960
	Growth differentiation factor 11 (Gdf11)	2.90	AF411599.2
	Otx2	2.74	AJ000939
Endocrine/Reproduction	Gonadotriphin-releasing hormone receptor 1 (GnRHR1)	2.37	AB092690.1
Immune	Complement factor (Bf/C2)	2.03	D84063
	Orla C3-1	2.47	AB025575
	Orla C4	2.23	AB02557
Metabolism	Cytochrome P450 1A	11.73	AY297923
	Transferrin	2.76	D64033
	Angiotensin converting enzyme (ACE)	2.23	L40175.1
	Proteasome activator 28 alpha subunit (PA28)	2.06	AF527990.1
	Procollagen lysine 2-oxoglutarate 5-dioxygenase precursor (PLOD)	2.05	AF054274.1
Nuc./Prot. binding	Translocon-associated protein γ-chain (Trap γ-chain)	2.96	AV669630
	Muscle-specific beta 1 integrin binding protein 2 (MIBP2)	2.57	BC066586.1
	Warm-temperature-acclimation-related-65 kDa-protein-like-protein (Wap65)	2.38	AB075199
	Heat shock protein 70 cognate	2.11	D13669.1
	Eukaryotic translation initiation factor 3, subunit 2 (beta)	2.07	BC029625.1
	Chaperonin containing TCP1, subunit 5	1.97	AY398321.1
Signal transduction	Annexin max 4	2.50	Y11255
	Receptor protein tyrosine phsphatase σ (RPTPσ)	2.23	AY369838.1
Non-categorized	Equilibrative nucleoside transporter 2 (ENT2)	3.06	AV670713
	Down-regulated		
Development	Wnt 10A	−2.56	AF359593
Endocrine/Reproduction	Estrogen receptor α	−2.17	AB033491
	Vitellogenin precursor	−2.22	AU179717
	Vitellogenin I	−2.78	AB064320
	Vitellogenin II	−4.34	AB074891
	Vitellogenin III precursor	−2.38	BM309828
	Choriogenin H	−3.12	D89609
	Choriogenin H minor	−4.17	AB025967
	Choriogenin L	−3.70	AF500194
Immune Metabolism	MHC Class I	−1.92	BA000027.2
	Chymotrypsin precursor	−5.26	AU179002
	Histone deacetylase 1	−1.85	BC085375.1
	RNA (guanine-9-) methyltransferase	−1.92	BC090650.1
	Angiotensin II receptor, type 1 (Agtr1)	−1.96	BC097125.1
Nuc./Prot. binding	Nuclear receptor related 1 (NURR1)	−2.44	AJ278700

*FD: Fold difference

tion, and proposed to have tumor suppressor activity, was shown to be regulated by HDAC1 in mouse[21]. In this study, the transcript level of HDAC1 was down-regulated to 1.85-fold after BaP exposure (Table 1). This result suggests BaP-induced tumors might occur through the attenuation of the gene regulatory function of HDAC1.

Endocrine Disruption

The estrogen receptor (ER) is a member of the steroid /thyroid hormone nuclear receptor superfamily of ligand-activated transcription factors[22]. Three types of ERs (ERα, ERβ, and ERγ) were found to be differentially expressed during sex determination and the changes in their transcription induced by endocrine disrupt-

ing chemicals (EDCs) in various teleost fish species were characterized[23,24]. The transcript levels of ERα decreased 2.17-fold in the microarray experiment (Table 1). Many EDCs have estrogenic activity and can bind to the ER, forming the EDC-ER complex. The subsequent binding of the EDC-ER complex to estrogen-responsive elements (ERE) in DNA results in the transcriptional activation of the vitellogenin genes[25].

Vitellogenin (Vtg) is an egg yolk precursor protein expressed only in female fish, as it is dormant in male fish under normal conditions. This egg yolk protein is synthesized in liver and accumulated and stored in growing oocytes as the food reserve of developing embryos. Vtg has been widely introduced in ecological toxicology as a powerful biomarker for the feminization of male fish, which is induced by endocrine disrupting chemicals (EDCs)[26]. Previously, the estrogenic effects of PCBs, inducing those of Vtg expression at the transcriptional and translational levels, have been observed in fish species[27]. In this study, the transcription of medaka Vtg related genes, such as Vitellogenin precursor (2.22-fold), Vitellogenin I (2.78-fold), II (4.34-fold) and Vitellogenin III precursor (2.38-fold), was found to be decreased in liver upon BaP exposure (Table 1).

Choriogenins are liver-derived precursor proteins located in the inner layer (zona radiate) subunits of the egg envelope (chorion) in Japanese medaka. Two types of choriogenins are recognized, one is choriogenin L (low-molecular weight protein) and the other is choriogenin H (high-molecular weight protein). Normally, both proteins are synthesized in the liver in response to estrogen in female fish. however, the induction of protein synthesis has been observed in male fish exposed to E_2 (17β-estradiol)[28]. The induction of choriogenin transcriptiol in male medaka liver upon treatment with endocrine disrupting chemicals, such as bisphenol A, nonylphenol and 17α-ethinylestradiol, has been demonstrated previously[29]. In this study, the induction of transcription upon BaP exposure was observed for three choriogenin genes in the microarray analysis (Table 1): Choriogenin H gene (3.12-fold), Choriogenie H minor gene (4.17-fold) and Choriogenin L gene (3.70-fold).

Gonadotrophin-releasing hormone (GnRH) has been known to stimulate the synthesis and release of gonadotrophic hormone in vertebrates, as well as growth hormone and prolactin in some fish species[30]. The specific receptor activated by GnRH is called GnRH receptor (GnRHR), a member of the seven transmembane G-protein coupled receptor (GPCR) family. Although GnRH is considered a neurohormone, the expression of GnRHRs has been detected in peripheral tissues related to the senses, reproduction and homeostasis, such as the eyes, olfactory epithelium, gonads, kidney, gut, liver and gills[30]. In our microarray analysis, GnRHR1 gene expression was observed to be upregulated 2.37-fold in BaP-exposed fish liver (Table 1).

Circulatory Problems

Angiotensin converting enzyme (ACE) is a circulating exopeptidase that catalyses the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor[31]. In the present study, ACE transcript levels were upregulated 2.23-fold in BaP-exposed fish (Table 1). Overexpression of ACE might cause increased synthesis of angiotensin II as well as increased metabolism of bradykinin, a vasodepressor, thereby leading to high blood pressure and heart failure. On the contrary, decreased transcriptional levels (1.96-fold) of Angiotensin II receptor type 1 (Agtr1), which mediates the functions of Angiotensin II[32], were observed in BaP-exposed fish (Table 1). This might a compensation response to the angiotensin II-induced high blood pressure.

Immune Responses

The complement system is a biochemical cascade, part of the large immune system, which helps remove invading bacteria and viruses. Complements, synthesized mainly in liver, play roles in the cytolysis of heterogeneous microorganisms, opsonization, the binding to specific complement receptors on immune system cells, and the removal of immune complexes from the immune system. Among the 20 proteins and protein fragments that comprise the complement system, bf /C2 promotes the specific cleavage of C3 for release of the biologically active fragments C3a and C3b. Component C4 is a component of the C3 and C5 convertases of the classical complement pathway[33]. The complement system might have roles in many diseases with immune deficiencies, and are also becoming increasingly implicated in neurodegenerative diseases such as Alzheimer's disease. In our transcription quantitative analyses, the gene expression levels of three complement component genes, Bf/C2, C3-1 and C4, were shown to be upregulated by approximately 2.03-, 2.47- and 2.23-fold, respectively (Table 1). The results indicate that BaP exposure might induce immune deficiencies and/or neurodegeneration.

Warm temperature acclimation-related protein 65-kDa (Wap65) has been shown to have homology with rat hemopexin. In medaka fish, two types of Wap65 (mWap65-1 and mWap65-2) have been identified and characterized[34]. Cold and warm temperature acclimation did not induce significant changes in the expression level of either gene, whereas the mRNA level of hemopexin-like protein or a Wap65 homologue gene was found to be significantly increased in fish species

under stressed situations, such as hypoxia[35]. A significant increase of *mWap65* transcription was observed in the livers of BaP-exposed medaka fishes (2.38-fold) (Table 1). Wap65 is also known to have functions in the immune response[36]. Thus, it is suggested that the immune system of medaka fish is severely damaged by BaP exposure.

The major histocompatibility complex (MHC) is a large gene family found in most vertebrates, and is functionally involved in both the adaptive and innate immune response[37]. BaP-exposed fish showed lower levels of MHC class 1 gene expression (1.92-fold) compared to that of control group (Table 1). Therefore, the negative effects of BaP on the immune system of living organisms are recognized.

Detoxification

Cytochrome P450 (CYP1A) is responsible for the metabolism of many xenobiotic compounds, pesticides and petroleum products. Induction of CYP1A mRNA by organic pollutants has been widely reported in fish species[38]. Conversely, the repression of CYP1A (CYP1A1 and CYP1A2) transcription due to oxidative stress has also been observed in hepatocytes[39]. In the microarray experiment, Cytochrome P450 1A was detected and its expression was found to be increased by 11.73-fold upon BaP exposure (Table 1).

Transferrin (Tf) is a glycoprotein, mainly synthesized in the liver and secreted into the blood, that plays a pivotal role in iron metabolism. Recently, induction of Tf mRNA expression in liver after cadmium exposure was found in Croceine croaker[40]. It was then assumed that increased levels of serum iron by cadmium exposure and/or increased rate of erythropoiesis might induce Tf mRNA transcription. We found that BaP exposure increased the transcript level of Tf to 2.76-fold in liver tissue (Table 1). The same scenario as the croaker case was observed in Ba-exposed marine medaka.

Molecular Chaperones

The Hsp70 family is a set of highly conserved proteins that assist protein-folding processes, guide translocation proteins across cellular organelle membranes, disassemble oligomeric protein structures and facilitate proteolytic degradation of unstable proteins. Hsp 70 chaperones are found in various cellular compartments and are induced by a variety of biological stresses[41]. In our microarray analysis, the transcript level of the Hsp70 mRNA was increased 2.11-fold upon BaP exposure (Table 1).

Chaperonin tailless complex polypeptide 1 (TCP1) ring complex (TRiC) is a heterooligomeric complex that facilitates the proper folding of many cellular proteins. This molecular chaperone plays an important role in tubulin and actin biogenesis, both of which are essential to cytoskeletal maintenance and neurotransmitter trafficking as well as segregation distortion[42]. BaP-induced TCP1, subunit 5 transcription (1.97-fold) was observed in the heterologous hybridization experiment (Table 1).

Response to Hypoxia

Procollagen lysine 2-oxoglutarate 5-dioxygenase precursor (PLOD) is a key enzyme in collagen biosynthesis. PLOD mRNA expression was observed to be upregulated at low oxygen tension in a mouse hepatoma cell line[43]. We found that BaP exposure increased PLOD transcription by 2.05-fold in liver compared to unexposed control group (Table 1).

Neuronal Deficiencies

Receptor protein tyrosine phsphatases (RPTPs) are essential for growth cone function, axon growth and synaptic connections in the nervous system. The role RPTPσ plays in the regulation of axon growth has been investigated in mice retinal ganglion cells (RGCs)[44]. In this report, we found that RPTPσ plays an active role in inhibiting axon regeneration in the injured central nervous system of adults. The mRNA expression level of RPTPσ was upregulated 2.23-fold in liver tissue of BaP-exposed fish (Table 1). The result suggests neuronal deficiency might occurr in BaP-exposed fishes.

Nuclear receptor related 1 (NURR1) is a transcription factor highly expressed in mesencephalic dopaminergic neurons of the midbrain[45], and it plays a role in the maintenance of the dopaminergic system of the brain. The transcription of NURR1 was decreased by 2.44-fold upon BaP exposure (Table 1).

Other Biological Responses

Proteasome activator 28 (PA28) activates the hydrolysis of small non-ubiquitinated peptide by binding to and activating the 20 S proteasome. PA28 has two homologous subunits, PA28α and PA28β The carboxyl terminus of the α subunit is necessary for PA28 to bind to the proteasome as well as proteasome activation[46]. In the present study, the expression of the medaka homolog of the PA28α gene was found to be upregulated 2.06-fold in BaP-exposed fish (Table 1). This might indicate that BaP exposure leads to proteasome activation through an increase in the PA28α transcription.

Translocons function in transporting eukaryotic proteins across the endoplasmic reticulum (ER) membrane. A Translocons complex is composed of four membrane integral subunits, α, β, γ and δ, termed translocon-asso-

ciated proteins or TRAPs. TRAPs complex plays an essential function in providing an aqueous protein-conducting channel that spans the membrane bilayer[47]. However, the precise function and mechanism of the TRAPs complex is yet not fully understood. In this study, the expression of Trap γ-chain, one of four subunits of the translocon, was increased 2.96-fold in microarray analysis (Table 1). Thus, the positive effect of BaP exposure on protein transport in marine medaka fish liver was speculated, though the exact mechanism requires further study.

Muscle-specific β1 integrin binding protein (MIBP) family is expressed in differentiating skeletal muscle and mature muscle cells, and integrins of the β1 family are suggested to play key regulatory roles in muscle development. Overexpression of MIBP in C2C12 myoblast cells resulted in the suppression of myogenic fusion and terminal differentiation[48]. In our interspecies microarray analysis, MIBP2 mRNA expression was increased in BaP-exposed fish by 2.57-fold relative to control (Table 1). The result suggests myogenic malformation might be induced by BaP exposure.

The members of equilibriative nucleoside transporter (ENT) family play an important role in the transport of nucleosides across the cell membrane[49]. ENTs also influence physiological processes ranging from cardiovascular activity to neurotransmission. ENT2 gene expression has been shown to become repressed during hypoxic condition[50]. In this study, however, the transcript level of ENT2 was significantly increased by 3.06-fold in BaP-exposed fish relative to the control group (Table 1). The reason for increased ENTs gene transcription upon BaP exposure remains unclear.

In this study, differentially expressed genes were profiled in marine medaka liver after BaP exposure by means of cross-species medaka cDNA microarray. The initial data obtained here will allow the subsequent studies to understand the molecular mechanisms of BaP toxicity and the promotion of systems biology approach to the organic chemical induced biological stress.

Materials & Methods

Animals, Exposure to Chemicals

Six to twelve month old marine medaka fish (*O. javanicus*) were fasted for 2 days and then exposed to BaP (Supelco, USA) dissolved in 0.1% dimethyl sulfoxide (DMSO) (100 ppb) for 24 hr. After rendering the animals unconscious by cold shock, the liver was excised and total RNA extracted. Three individuals were assigned to an experimental group, with their pooled RNAs used for one experiment. A group exposed to 0.001% DMSO was used as the control group.

Total RNA Extraction and Preparation of cDNA Probes

An RNeasy Mini Kit (QIAGEN, Inc, Valencia, CA, USA) was used for the extraction and purification of medaka hepatic total RNA, with 1 μg each of the extracted total RNA reverse-transcribed using T7-oligo dT primers to synthesize single-stranded cDNA. Double-stranded cDNA was synthesized from the single-stranded cDNA, followed by *in vitro* transcription with amino-allyl UTP to generate amino-allyl labeled aRNA target samples. The aRNA samples were purified, coupled with amine reactive fluorescent dye, Cy5, and then re-purified for hybridization on the medaka cDNA microarray. All processes from reverse-transcription of total RNA to Cy5-aRNA synthesis were performed with an Amino Allyl MessageAmp aRNA kit (Ambion Inc, Austin, TX, USA).

cDNA Microarray and Gene Expression Analysis

The cDNA microarray used in this study was fabricated by Ecogenomics, Inc. (Fukuoka, Japan), and contained 833 adult and 2,222 embryonic medaka cDNA gene probes. Detailed information on these gene probes are listed at the following web sites: http://www.ecogenomics.co.jp/Medaka750_GeneFunction_Sept2006.pdf; http://www.ecogenomics.co.jp/Ol_Egg_EGArray_2222GeneList.pdf. Each of the labeled target samples were hybridized on two cDNA microarrays. Hybridizations of the labeled target samples and gene probes on the microarray were performed for 16 hours at 42˚C in 45 μL of 50% formamide (Wako, Osaka, Japan)/5× SSC (SIGMA, St. Louis, MO)/0.5% SDS (Ambion Inc, Austin, TX) hybridization solution in a moisture chamber, followed by post-hybridization washing (two washes in 1× SSC/0.2% SDS at 42˚C for 5 minutes and 15 minutes, two washes in 0.1× SSC/0.2% SDS at ambient temperature for 5 minutes each, and a final two washes in 0.1× SSC at ambient temperature for 2 minutes each). The prepared microarray slides were scanned using a GenePix 4000B scanner (Molecular Devices, Sunnyvale, CA, USA) using a resolution of 10 μm.

Statistical Analysis

For statistical analysis, the raw microarray data collected by the GenePix 4000B scanner were independently normalized to the median expression value of each in the microarray. Data were then integrated into the ArrayStat z-test (Imaging Research Inc, St. Catharines, ON, Canada), with a significance determination of $P < 0.01$, to obtain the differential gene expression

ratio (also expressed "fold differenc" or "FD") for each of the gene probes on the microarray. The FD values were calculated by taking the ratio between the average of six signal strengths (3 spots × 2 microarrays) for the control group and the average of six signal strengths (3 spots × 2 microarrays) for the BaP-exposed experimental group.

Acknowledgements

This subject is supported by Korea Ministry of Environment as "Eco-technopia 21 project" (KORDI Project No. PN64600), and the Korea Ocean Research & Development Institute project (PE98313) to SY; the grant from the Ministry of Education, Science and Technology to HSP (2007-04269).

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