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ORIGINAL PAPER

Transcriptome information of the Arctic green sea urchin and its use in environmental monitoring

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Abstract The Arctic sea urchin *Strongylocentrotus droebachiensis* is considered to be a potential indicator species for assessing aquatic environmental conditions in the polar region. To develop a gene resource for the Arctic sea urchin, we sequenced *S. droebachiensis* cDNAs using a GS-20 sequencer and obtained expressed sequence tags information. In the assembly stage, 31,526 transcripts were obtained and showed a 32 % hit rate in the GenBank non-redundant database with 9,995 unigenes. Of the transcripts obtained in this species, we found several stress- and cellular defense-related genes (antioxidant-related genes and genes from the heat shock protein family) that are useful for the monitoring of pollutant-triggered stress responses at the molecular level. To validate the usefulness of these potential biomarker genes, we analyzed the transcript profiles of selected genes in response to polychlorinated biphenyls (PCB) mixtures (Aroclor 1254) for 48 h. PCB contamination is a present-day threat to the health of individual organisms and ecosystems in the polar region. We showed that 11 of 14 genes responded to PCB treatment at transcriptional levels, with the most dramatic upregulation observed in the *hsp70.5* gene. In this paper, we summarize the expressed cDNA information and discuss its potential use in ecotoxicological genomic studies on PCB exposure.

Keywords Arctic sea urchin · *Strongylocentrotus droebachiensis* · Expressed sequence tag · Polychlorinated biphenyls · Aroclor 1254

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Introduction

The sea urchin has been used for the environmental monitoring of marine environments in addition to its wide use as a model species for studies in developmental biology. In the genus *Strongylocentrotus*, the Arctic green sea urchin *S. droebachiensis* is an important species that can be found in shallow rocky subtidal substrata in Kongsfjorden on West Spitsbergen, Svalbard (Hop et al. 2002; Beuchel and Gulliksen 2008). For reliable environmental monitoring, a collection of massive gene information would be helpful in the bio-monitoring of aquatic ecosystems and field toxicity tests through an elucidation of the molecular defense mechanisms of these species in diverse environmental conditions. New attempts for coordinated approaches in environmental toxicogenomics and proteomics are being developed in the laboratory and in the field.

The Arctic Ocean is an extreme environment surrounded by continents and holds unique and genetically diverse life forms that are highly adapted for successful survival and reproduction in this harsh environment, including sponges, polychaetes, amphipods, pycnogonids, ascidians, bryozoans, mollusks, isopods, and fishes (Fogg 1998). Recently, increased levels of various types of pollutants have worsened environmental pollution (Bard 1999; Muir et al. 1999). Thus, increased heavy metals, organic materials, and other pollutants are threatening arctic animals through the accumulation of toxic substances in water, sediment, and animal tissues (Wagemann et al. 1996; Fant et al. 2001; Dehn et al. 2006). Of the pollutants entering the Arctic Ocean, some chemicals such as chlorinated hydrocarbons [e.g., polychlorinated biphenyls (PCBs)], polychlorinated camphenes (PCCs, toxaphene), and organochlorine pesticides (DDT and its metabolites) have been detected in higher trophic levels of organisms (Muir et al. 1988; Oehme 1991). Generally, the majority of PCBs are transported via water runoff and atmosphere, but they can also accumulate in the land or ocean for a long time and be slowly released into the water or food web.

The structural properties of several PCBs allow them to act as an agonist of the aryl hydrocarbon receptor (AhR), resulting in cellular toxicity, including some neurotoxic and immunotoxic effects (Giesy and Kannan 1998; Van den Berg et al. 1998). Moreover, most PCBs are very stable and do not readily decompose in the natural environment. Due to the highly lipophilic and persistent properties of chlorinated hydrocarbons, most PCBs accumulate in fatty tissues through the Arctic food web by efficiently being transferred from zooplankton to top predators (Skaare et al. 1990; Gabrielsen et al. 1995). However, most data on the correlation between PCB levels and their adverse effects have focused on vertebrate species such as glaucous gulls, northern fur seals, and polar bears that live at the top of the food chain (AMAP 2004). Therefore, there is a strong need for research to understand the detrimental effects of PCBs on Arctic invertebrates.

To understand the molecular responses to environmental pollutants, the gene mining of certain animals is necessary to integrate gene modulation into mechanistic insights. The collection of expressed sequence tags (ESTs) is an efficient method to sequence unknown transcripts or transcriptomes in a short amount of time with less effort. Much information can easily be obtained in terms of the gene expression, specific gene functions, and their protein products (Adams et al. 1991). Recently, next generation sequencing (NGS) technology has been widely used to obtain massively parallel transcript information. Linkages between gene/protein information and its application to biomarker development are important parameters in the new toxicological toolbox, as environmental chemicals directly affect genomic and proteomic responses in organisms. The availability of EST information coupled with genomics can accelerate the understanding of the link between gene and protein expression in organisms after stimuli from diverse environmental pollutants. Therefore, EST information could be a reliable early signal to evaluate aquatic environmental pollution.

The sea urchin *S. purpuratus* has been developed as a model organism for developmental and ecotoxicological approaches via extensive research from molecular to ecological levels (Vega and Epel 2004; Goldstone et al. 2006; Hamdoun and Epel 2007; Bosnjak et al. 2009). However, other sea urchins in the genus *Strongylocentrotus* have not been used to date as potential indicators for environmental monitoring. In this study, we report the construction of an EST database of the Arctic green sea urchin *S. droebachiensis* to obtain useful genetic information for Arctic environmental studies, as it has a circumpolar distribution, extending down to the Western North Atlantic (41°N) and the Western North Pacific (36°N). These data will be helpful in obtaining a better understanding of the molecular basis of the responses of *S. droebachiensis* to various environmental challenges through a genomic approach (e.g., microarray development or additional RNA sequencing applications).

Materials and methods

Strongylocentrotus droebachiensis

The Arctic green sea urchins were collected from shallow subtidal water (5–15 m) at Kongsfjorden (79°N, 12°E) where there is a glacial fjord that is influenced by both Atlantic and Arctic water containing a mixture of boreal and Arctic flora and fauna (Hop et al. 2002). They were acclimated in a 40 L container in a temperature-controlled aquarium at 1.0 ± 0.1 °C under a constant air supply for 5 days. The species identity was checked by *COI* sequence analysis of the mitochondrial DNA (Rhee et al. 2012a, b).

Total RNA extraction and single-strand cDNA synthesis

Pooled tissue from the esophagus, gonad, radial canal, and muscle ($n = 5$) were homogenized in three volumes of TRIZOL® reagent (Molecular Research Center, Inc.) with a tissue grinder and stored at –80 °C until use. Total RNA was isolated from the tissues according to the manufacturer's instructions. Genomic DNA was removed using DNase I (Sigma, St. Louis, Mo). After DNase I treatment, total RNA was purified using the RNeasy Mini Spin Column (Qiagen, Germantown, MD). The quantity of total RNA was measured at 230, 260, and 280 nm with a

spectrophotometer (Ultrospec 2100 pro, Amersham Bioscience). To check for genomic DNA contamination, we loaded the total RNA in a 1 % agarose gel that contained ethidium bromide (EtBr) and visualized this on a UV transilluminator (Wealtec Corp.). To verify the total RNA quality, we loaded the total RNA onto a 1 % formaldehyde/agarose gel with EtBr staining and checked the 18/28S ribosomal RNAs integrity and band ratio. Single-strand cDNA was synthesized from total RNA using an oligo $(dT)_{20}$ primer for reverse transcription (SuperScript™ III RT kit, Invitrogen, Carlsbad, CA,USA).

Brief summary of cDNA library preparation, pyrosequencing, assembly, and gene ontology analysis

A cDNA library and pyrosequencing were conducted according to the manufacturer's instructions (Roche Applied Science, Genome Sequencer 20™ System). In the raw reads, highly repetitive and non-informative reads were excluded prior to the assembly process. In the assembly stage, we used a TGI clustering tool (TGICL, http://compbio.dfci.harvard.edu/tgi). Microbial genes were predicted with Glimmer 3.02 (Delcher et al. 2007) and were excluded. The assembled EST translations were aligned for gene prediction with the GenBank non-redundant (NR including all GenBank, EMBL, DDBJ, and PDB sequences except for the EST, STS, GSS, or HTGS) amino acid sequence database with BLASTX. Subsequently, the data were sorted by read length, gene annotation, GenBank number, E value, and closely matched species and their accession number (Online Resource 1). To increase the number of valid annotations, all of the aligned results were sorted by E value $\leq$1.00E-05 with a cut-off value >200 bp. In addition, after the cleaning process, we aligned *S. droebachiensis* reads directly with the transcriptome database of *S. purpuratus* (Tu et al. 2012; http://www.spbase.org/SpBase/rnaseq). Matched *S. droebachiensis* genes were represented with the number of the corresponding *S. purpuratus* gene (Online Resource 2). The Gene Ontology (GO) functional annotations and the KEGG pathway mapping were determined by the Blast2GO automated sequence annotation tool (http://www.blast2go.org). The three main categories—biological process, cellular component, and molecular function—were obtained using the default parameters. The assembly, gene annotation, and GO analysis were performed at the NICEM, Seoul National University (Seoul, South Korea).

PCB mixture exposure

To study the effect of PCB mixture (Aroclor 1254) exposure on stress- and cellular defense-related gene expression, *S. droebachiensis* ($n = 25$) were exposed to 1 parts-per-billion (ppb) of waterborne PCB mixture for 48 h. They were not fed during the experiment, and the water temperature was maintained at 1.0 ± 0.1 °C throughout the experimental period. The samples ($n = 5$) were collected at 0, 6, 12, 24, and 48 h. Methanol was used as the solvent control, with a concentration that was maintained at <0.001 %. The same numbers of *S. droebachiensis* were collected as control groups at each sampling time point.

Table 1 Primer information of the stress-related genes used in this study

Gene and accession number		Sequences (5′ → 3′)
GST alpha	F	AAACAACCAGGGCTTAAGGAGTACC
(JQ406603)	R	GCCATGACGATCACCAGTCCAG
GST mu	F	CATTCTGGACATCCTTAACGACAAC
(JQ406604)	R	AAGGTATTTGGCAATCTTCTCGTCG
GST omega	F	GAGATTTGGAAAGTTCATTGCAGG
(JQ406607)	R	TCTTGTTCCACGCTTTCAACATTC
GST pi	F	AACGAAGTAGAAGCCACACGCATC
(JQ406605)	R	CAGCCTTCTTAGCTTCGTCTTTCTC
GST theta	F	AATGATTGGTTGGCTGGAGATG
(JQ406606)	R	AAGGCCCTGAGTTTAGGTCTCCC
Microsomal GST	F	CTATGGTGGAGAGGGTCAGAAGATG
(JQ406602)	R	GTTGAAGCTCCCGAGGTGAGTG
Glutathione reductase	F	GAGTATGGAATCAGTAGTGATG
	R	CTTCCAAGTTCTTTCAGGATTCCTG
(JQ406610)		
MnSOD	F	TCCCGCTATTTGGTATTGATGTG
(JQ406608)	R	GTCGCTCCAGTTAGCAATGTTAAAG
CuZnSOD	F	GACGAAGAGAGACATGTTGGTGATC
(JQ317298)	R	CGTCCAATGATGGATTGTGGTC
Catalase	F	ACCGACCCAGACTACGCCATTC
(JQ406609)	R	CCTGCTCCTGGGTCATGATCTG
Hsp60	F	CAAGTCTCTTGCTGAACACAG
(JQ406611)	R	TCTCAGGGTCAACGTATGCCA
Hsp70.5	F	CAGCTGTTCAAGCTGCCATC
(JQ317300)	R	GTCTCGATACCCATGGAC
Hsc70	F	GTGCAAGGAAGTCTTGGATTG
(JQ317299)	R	GGTGATGATGGGTGCACAGAC
Hsp90	F	GCTGGTCAGGTTCTTCTCCTC
(JQ406612)	R	GTTCCAGCAACAAAGTAGATGAC
β-actin	F	GTCGCCATCCAGGCCGTGCTTTC
(AY618350)	R	GAAGGGCGTAACCCTCGTAGATGG

Real-time RT-PCR

To investigate the specific expression patterns of the genes of interest, real-time RT-PCR was performed. Each reaction included 1 μL of cDNA and a 0.2 μM primer (real-time RT-F/R and *β-actin* RT-F/R, GenBank no. AY618350), as

shown in Table 1. The primers were designed after comparing the exon/intron boundary with the genomic DNA using GENRUNNER software (Hastings Software, Inc, NY; USA) and confirmed by the Primer 3 program (Whitehead Institute/MIT center for Genome Research). To determine the amplicon identity, all of the PCR products were cloned into the pCR2.1 TA vector and sequenced with an ABI 3700 DNA analyzer (Bionics Co., Seoul, South Korea). The optimized conditions were transferred according to the following CFX96™ real-time PCR system protocol (Bio-Rad). The reaction conditions were as follows: 95 °C/3 min; 40 cycles of 95 °C/30 s, 55 °C/30 s, and 72 °C/30 s. To confirm the amplification of specific products, the cycles were continued to check the melting curve under the following conditions: 95 °C/1 min, 55 °C/1 min, and 80 cycles at 55 °C/10 s with a 0.5 °C increase per cycle. SYBR® Green (Molecular Probes Inc., Invitrogen) was used to detect the specific amplified products. Amplification and detection of the SYBR® Green-labeled products were performed using a CFX96™ real-time PCR system (Bio-Rad, Hercules, CA, USA). The data from each experiment were expressed relative to the expression levels of the *β-actin* gene to normalize the expression levels between the samples. All of the experiments were performed in triplicate. Data were collected as threshold cycle (C_T) values (PCR cycle numbers where the fluorescence was detected above a threshold and decreased linearly with increasing input target quantity) and were used to calculate the ΔC_T values of each sample. The change in the relative gene expression was calculated by the $2^{-\Delta\Delta c_T}$ method (Livak and Schmittgen 2001).

Statistical analysis

Data were expressed as mean ± SD. Significant differences were analyzed using one-way ANOVA followed by Tukey's test. $P < 0.05$ was considered significant. The SPSS version 17.0 (SPSS Inc., Chicago; IL, USA) software package was used for the statistical analysis.

Results

Establishment of the EST database

The pyrosequencing results from the Arctic green sea urchin *S. droebachiensis* are summarized in Table 2. Unexpectedly, a large number of raw reads were discarded due to highly repetitive and non-informative reads, resulting in 109,141 valid reads obtained from 184,541 raw reads. After a cleaning process, 31,526 transcripts were detected in the assembly stage. BLASTX searching of the NR database showed that 32 % of the transcripts were hits in the EST database. After all of the transcripts were compared with the GenBank NR amino acid sequence database using BLASTX, a total of 9,995 assembled transcripts with an E value ≤ 1.00E-05 were obtained as reliable annotation results (Table 2; Online Resource 1). The average read length of the hits was 194 bp, and the largest one was 1,839 bp. The assembled transcript information with the GenBank NR amino acid sequence database and BLASTX was deposited as supplementary material (Online Resource 1).

Table 2 Overview of the cDNA sequencing and assembly

# Raw reads	109,141
Sequence cleaning process[a]	
# Valid reads	92,388
# Trimmed reads of a valid process	8,952
# Trashed reads[b]	7,801
Lucy cleaning process	
# Valid reads	86,526
# Discarded reads	5,862
Assembly stage[c]	
# Transcripts[d]	31,526
# Hits[e]	9,995
% with hits[f]	32
Average read length of hits (bp)	194
Largest read length (bp)	1,839

[a] Minimum length: 50 base pair

[b] Trashed reads composed of short reads (7,254), low quality reads (262), short quality reads (208), and dust reads (77)

[c] Assembly stage 2 was assembled with the contigs and singletons form the assembly stage 1 without singlets using the TGI clustering tool (TGICL)

[d] total number of transcripts including singletons

[e] number of transcripts that have a BLASTX hit

[f] percent of stage 1 assembled transcripts that have a BLASTX hit

Gene ontology analysis

To classify the gene distribution based on putative functions, GO analyses were conducted on the *S. droebachiensis* database using Blast2GO software (Online Resource 3 to 5). Entire annotated reads were categorized as cellular components, molecular functions, or biological processes according to their functions within each ontology class. All GO terms for each category were adopted from level 2. The GO terms of each level from 3 to 9 were incorporated in the supplementary materials (Online Resource 3 to 5). In the different GO categories, the cellular components were classified into seven sub-categories (Fig. 1a), which are the major proteins composed of cellular (33 %), organelle (28 %), and macromolecular complexes (20 %). Based on molecular function, they fell into nine categories (Fig. 1b), and proteins with GO binding (39 %) and catalytic activity

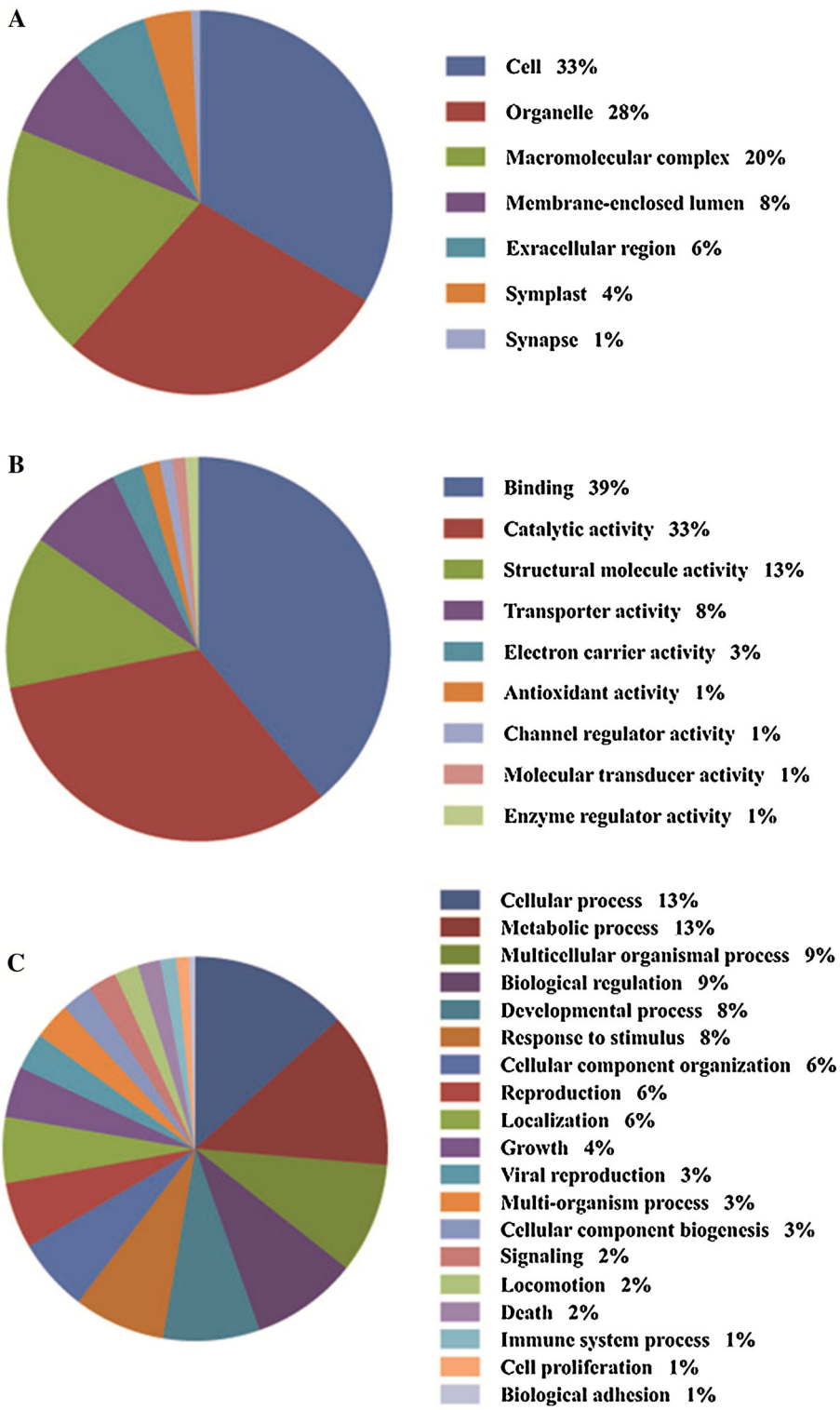

Fig. 1 Categories of gene ontology for *S. droebachiensis* ESTs. **a** Molecular function, **b** cellular components, **c** biological process

(33 %) were the most enriched, followed by proteins involved in structural molecule activity (13 %) and transporter activity (8 %). In addition, 1 % of the proteins in the molecular function category showed antioxidant activity. Finally, 19 categories were classified as biological processes (Fig. 1c). In the biological process category, most of the represented genes were involved in cellular (13 %), metabolic (13 %), multicellular organismal (9 %), or developmental process (8 %), or biological regulation (9 %).

In addition, we assigned all of the assembled genes to functional classifications based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (Table 3; Online

Table 3 Most highly represented KEGG classifications in *S. droebachiensis* transcripts

Classification	#	%
Metabolic pathways	130	22.8
Oxidative phosphorylation	68	11.9
Nitrogen metabolism	57	10.0
Biosynthesis of secondary metabolites	34	6.0
Arachidonic acid metabolism	29	5.1
Purine metabolism	23	4.0
Methane metabolism	13	2.3
Phenylalanine metabolism	12	2.1
Metabolism of xenobiotics by cytochrome P450	11	1.9
Glutathione metabolism	10	1.8
Cysteine and methionine metabolism	9	1.6
Drug metabolism—cytochrome P450	9	1.6
Phenylpropanoid biosynthesis	9	1.6
Glycine, serine and threonine metabolism	7	1.2
Citrate cycle (TCA cycle)	6	1.1
Glycerolipid metabolism	6	1.1
Tryptophan metabolism	6	1.1
Aminobenzoate degradation	5	0.9
Glycerophospholipid metabolism	5	0.9
Glyoxylate and dicarboxylate metabolism	5	0.9
Starch and sucrose metabolism	5	0.9
Arginine and proline metabolism	4	0.7
Carbon fixation pathways in prokaryotes	4	0.7
Glycolysis/gluconeogenesis	4	0.7
Pantothenate and CoA biosynthesis	4	0.7
Retinol metabolism	4	0.7
Valine, leucine and isoleucine biosynthesis	4	0.7
Amino sugar and nucleotide sugar metabolism	3	0.5
Ascorbate and aldarate metabolism	3	0.5
Butanoate metabolism	3	0.5
Drug metabolism—other enzymes	3	0.5
N-Glycan biosynthesis	3	0.5
Pyrimidine metabolism	3	0.5
Pyruvate metabolism	3	0.5
Sphingolipid metabolism	3	0.5
Steroid hormone biosynthesis	3	0.5
Valine, leucine and isoleucine degradation	3	0.5
Various types of *N*-glycan biosynthesis	3	0.5
Alanine, aspartate and glutamate metabolism	2	0.4
α-Linolenic acid metabolism	2	0.4
Benzoate degradation	2	0.4
β-Alanine metabolism	2	0.4
Carbon fixation in photosynthetic organisms	2	0.4
Cyanoamino acid metabolism	2	0.4
Fatty acid elongation	2	0.4
Fatty acid metabolism	2	0.4
Glycosaminoglycan degradation	2	0.4
Lysine degradation	2	0.4
Other types of O-glycan biosynthesis	2	0.4
Pentose and glucuronate interconversions	2	0.4
Porphyrin and chlorophyll metabolism	2	0.4
Propanoate metabolism	2	0.4
Bisphenol degradation	1	0.2
Caprolactam degradation	1	0.2
Ether lipid metabolism	1	0.2
Ethylbenzene degradation	1	0.2
Folate biosynthesis	1	0.2
Galactose metabolism	1	0.2
Geraniol degradation	1	0.2
Inositol phosphate metabolism	1	0.2
Isoquinoline alkaloid biosynthesis	1	0.2
Limonene and pinene degradation	1	0.2
Linoleic acid metabolism	1	0.2
Novobiocin biosynthesis	1	0.2
One carbon pool by folate	1	0.2
Other glycan degradation	1	0.2
Phenylalanine, tyrosine and tryptophan biosynthesis	1	0.2
Phosphatidylinositol signaling system	1	0.2
Primary bile acid biosynthesis	1	0.2
Steroid biosynthesis	1	0.2
Styrene degradation	1	0.2
Sulfur metabolism	1	0.2
Synthesis and degradation of ketone bodies	1	0.2
Taurine and hypotaurine metabolism	1	0.2
Terpenoid backbone biosynthesis	1	0.2
Thiamine metabolism	1	0.2
Toluene degradation	1	0.2
Tropane, piperidine and pyridine alkaloid biosynthesis	1	0.2
Tyrosine metabolism	1	0.2

Resource 6). As shown in Table 3, the "metabolic pathways" category was the most represented among the *S. droebachiensis* transcripts (22.8 %). The overall classification showed the different distributions and compositions of different gene sets with their putative functions. Several classifications were considered for environmental monitoring, such as "Metabolism of xenobiotics by cytochrome P450'', "Glutathione metabolism", "Drug metabolism-cytochrome P450'', and other metabolic pathways involved in the degradation of organic and inorganic compounds. In this category, we obtained potentially useful biomarker genes such as oxidative stress-related genes and heat shock proteins for monitoring that are closely associated with the environment of the polar region.

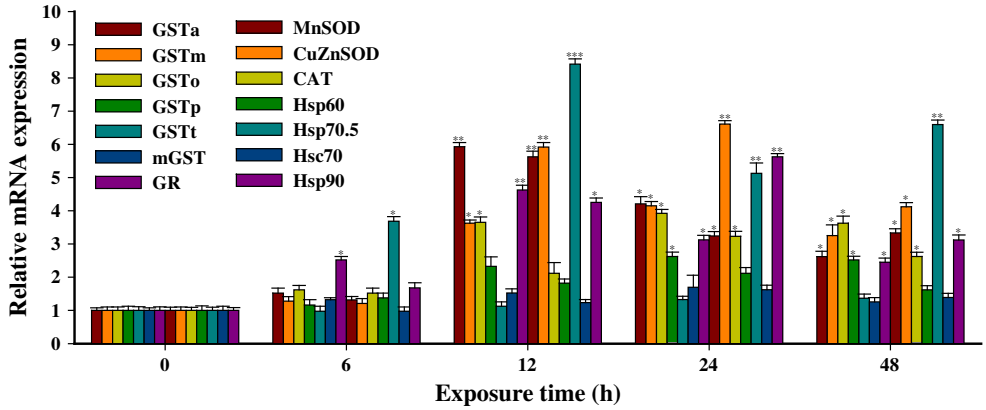

Fig. 2 Time-course effects of 10 ppb of PCB mixture (Aroclor 1254) on *S. droebachiensis* mRNA expression at different sampling time points (0, 6, 12, 24, and 48 h). The transcriptional expressions are shown relative to *β-actin,* which was used as the reference housekeeping gene. Data are the mean ± SD of three replicates of the exposed sea urchins. The symbols (*, **, and ***) indicate $P<0.05$, $P<0.01$, and $P<0.001$, respectively

Application of the transcript profile analysis to PCB mixture (Aroclor 1254) exposure

With NGS technology, we obtained several antioxidant-related genes from the Arctic green sea urchin *S. droebachiensis*: *GST alpha* (*GSTa*) (JQ406603), *GST mu* (*GSTm*) (JQ406604), *GST omega* (*GSTo*) (JQ406607), *GST pi* (*GSTp*) (JQ406605), *GST theta* (*GSTt*) (JQ406606), *microsomal GST* (*mGST*) (JQ406602), *glutathione reductase* (*GR*) (JQ406610), *Mn-SOD* (*MnSOD*) (JQ406608), *Cu/Zn-SOD* (*CuZnSOD*) (JQ317298), and *catalase* (*CAT*) (JQ406609). As a persistent organic pollutant, the PCB mixture treatment modulated the transcriptional expression of several *GSTs* of *S. droebachiensis* in this study (Fig. 2). Of the GST isoenzymes, the transcripts of *GSTa*, *GSTm*, *GSTo*, and *GSTp* were significantly upregulated ($P < 0.05$ to $P < 0.001$) by 1 ppb of PCB mixture for 48 h, but no significant effect was observed on *GSTt* and *mGST* at each time point. Other oxidative stress-related genes, *GR*, *MnSOD*, and *CuZnSOD*, also increased significantly after exposure to 1 ppb of PCB mixture for 48 h in *S. droebachiensis* (Fig. 2).

Despite the importance of heat shock proteins (hsps) on the environmental monitoring of the polar region, there is no information available on genes or proteins in this species. In this study, we annotated four *hsp* genes: *hsp60* (JQ406611), *hsp70.5* (JQ317300), *hsc70* (JQ317299), and *hsp90* (JQ406612) from the Arctic green sea urchin and tested their modulated pattern after exposure to a PCB mixture. The mRNA expression of *S. droebachiensis hsp70.5* and *hsp90* were significantly upregulated ($P < 0.05$ to $P < 0.001$) by 1 ppb of PCB mixture for 48 h, but no significant effect was observed on *hsp60* and *hsc70* at each time point (Fig. 2).

Discussion

Next generation sequencing technology has proven to be a powerful tool for discovering ESTs that are valuable gene sets in a particular organism (Margulies et al. 2005). To date, pyrosequencing-based EST identification has been successfully performed for diverse animals, but these platforms have not yet been applied in the Arctic green sea urchin *S. droebachiensis*. Previously, traditional methods such as the construction of cDNA libraries have been performed using the Gibco P-Sport vector for sea urchins. Lee et al. (1999) reported identifying 956 ESTs from 7 h (mid-cleavage) embryos of the sea urchin *S. purpuratus*. Subsequently, 20,000 unique ESTs were annotated in 107,283 cDNA clones in the same sea urchin species (Poustka et al. 2003), and approximately 7,000 total ESTs were annotated that were associated with embryo development. In 2006, the *S. purpuratus* genome encoding about 23,300 genes in the embryogenic transcriptome was sequenced using a combined strategy with a whole-genome shotgun approach and bacterial artificial chromosome (BAC) library analysis (Sea Urchin Genome Sequencing Consortium 2006; Samanta et al. 2006). Based on its genome information, *S. purpuratus* has been extensively used in genomic studies. Goldstone et al. (2006) made a genome-wide survey of the chemical defense transcriptome that participates in responses to environmental stressors. Temporal transcriptional profiles of 35,282 genes were analyzed in the *S. purpuratus* embryo (Wei et al. 2006). Also, extensive tissue-specific expression profiles were investigated for the study of gene regulatory networks (Poustka et al. 2007). Recently, temporal and spatial transcript expression profiles of ATP-binding cassette (ABC) transporters were reported in the *S. purpuratus* embryo

(Shipp and Hamdoun 2012). About 7,000 different annotated transcripts in 24 functional classes were analyzed from different developmental stages (10 different embryonic stages, six feeding larval and metamorphosed juvenile stages) and tissues of *S. purpuratus* (Tu et al. 2012, 2014). Based on this genomic information, *S. purpuratus* has been highlighted as an important model animal for evolutionary and developmental research. Although EST identification and genome resource research has focused on *S. purpuratus*, we believe that the sea urchin *S. droebachiensis* can be a good model animal in certain areas such as polar regions.

In this study, we constructed an EST database of the Arctic green sea urchin *S. droebachiensis* to obtain useful gene information for future environmental studies assessing the molecular basis of the animal's defense system against various environmental challenges and marine contamination in the Arctic region. Overall, the GS-20 platform-based pyrosequencing results of the Arctic green sea urchin *S. droebachiensis* was similar to previous results obtained using the bacterium *Mycoplasma genitalium* (110 bp) (Margulies et al. 2005), the hermaphroditic fish *Kryptolebias marmoratus* (116–129 bp) (Rhee et al. 2011), and the rockshell *Thais clavigera* (114 bp) (Rhee et al. 2012a, b) in terms of the sequencing quality and the average read length of views of the transcripts. Although the average read length of the contigs was short compared to those of recent NGS products due to the limited ability of the initial model sequencer GS-20, these results serve to increase the available cDNA sequence information of the Arctic green sea urchin for studying the marine ecology and environment of the polar regions.

GO/KEGG analysis suggested that this kind of survey of *S. droebachiensis* may be helpful in identifying novel genes with the proper gene annotation to be involved in diverse molecular pathways. Moreover, this GO information may offer advantages in interpreting large gene expression profiles in microarray applications or other genomics analyses (e.g., RNA Seq). Taken together, this EST profiling is of value in the identification of specific genetic information, indicating that this approach would be helpful to mine important genes in relation to defense mechanisms in a short period of time. To validate the usefulness of the genetic information of *S. droebachiensis* for environmental monitoring, we chose several biomarker genes that retain strong biomarker potential in diverse environmental conditions and tested their transcriptional changes in response to PCB mixture (Aroclor 1254) exposure.

The bioaccumulation of organic compounds such as PCB mixtures threatens the ecosystem of the polar regions through diverse routes through the food web (Wania and Mackay 1993; Kumar et al. 2002). These pollutants can induce various cytotoxic stresses, including oxidative stress that adversely affects the macromolecules within aquatic organisms (Goerke et al. 2004). Marine invertebrates have developed several intracellular defense systems consisting of complex enzymes as protective mechanisms (Goldstone et al. 2006; Yadetie et al. 2012; Kim et al. 2013). Therefore, analysis of the molecular defense mechanisms of marine benthos has been considered to be a promising approach for marine environmental monitoring. However, despite their wide distribution, there is little molecular information on polar animals such as the Arctic green sea urchin (Abele and Puntarulo 2004; Verde et al. 2006; Nahrgang et al. 2009; Noël et al. 2014), although the functions of several enzymes involved in the molecular defense system have been well-characterized in a variety of marine animals. Therefore, assessing the genetic information of the polar sea urchin and characterizing its expression profile would be important and useful in revealing the mechanisms behind these animals' molecular response to environmental pollutants in polar field sites as well as under laboratory conditions.

Although oxidation is crucial for animal life, certain levels of reactive oxygen species (ROS) can cause oxidative stress and cellular damage. To diminish the detrimental effects induced by oxidation, endogenous antioxidant enzymes including glutathione *S*-transferase (GST),GR, superoxide dismutase (SOD), and CAT play a pivotal role in the antioxidant defense system by scavenging free radicals and ROS generated by various factors as well as by normal metabolism in oxygen-consuming organisms (Hayes et al. 2005; Blanchette et al. 2007). Intracellular ROS produces hydrogen peroxide (H_2O_2), hypochlorous acid (HClO), and free radicals such as the hydroxyl radical (·OH) and the superoxide anion (O_2^-). Glutathione (GSH)-related antioxidant enzymes detoxify these electrophilic compounds to prevent the propagation of free radicals (Sies 1999).

The induction or suppression of GSTs has shown promise as specific biomarkers in the biomonitoring of aquatic pollution in diverse marine invertebrates (Hyne and Maher 2003; Durou et al. 2007; Martín-Díaz et al. 2007; Rhee et al. 2007). PCB and related-organic compounds have a long half-life due to their stability and can subsequently accumulate in animals and result in detrimental cytotoxic effects on cells. The uptake of PCBs into animals triggers several biochemical responses in phase I and II detoxification systems. In particular, GST is the most important detoxification enzyme in the phase II metabolism as well as in the antioxidant defense system. In *S. droebachiensis*, we found that PCB mixture exposure modulated the transcripts of *GST* isoenzymes for metabolizing the toxicants, as shown in Fig. 2.

Previously, in the liver tissues of juvenile rainbow trout, Machala et al. (1998) showed that the activity of the

cytosolic GST protein was induced in response to PCB 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) exposure. Long-term exposure to PCB mixtures also induced GST activities in the liver of juvenile rainbow trout (Blom and Förlin 1998). Thus, the activity of certain GST proteins may be considered potential biomarkers for exposure to PCB mixtures (Aroclor 1254), as shown in the Atlantic eel *Anguilla anguilla* (Pérez-López et al. 2002a). In addition, several different GST isoenzymes showed significant induction by the same PCB mixture in the liver tissues of rainbow trout (Pérez-López et al. 2002b). In the Antarctic bivalve *Laternula elliptica*, 0.1 and 10 ppb of the PCB mixture (Aroclor 1254) significantly induced the mRNA expression of *GSTr*, *GSTp*, and *GSTs* for 48 h (Park et al. 2009a). Even though these observations were mainly focused on the detection of indicators of oxidative stress, the in vitro result suggests that PCBs could induce an increase in ROS at steady state levels (Hennig et al. 2002). PCBs and their metabolites could also induce cellular oxidative stress by altering the electron transfer in mitochondrial electron transport chains (Turrens et al. 1985). Therefore, metabolic changes in the intracellular ROS level and/or mitochondria would lead to excess oxidative stress during PCB exposure in the Arctic green sea urchin. Given the fact that PCBs have significant effects on the oxidative metabolism leading to ROS production, each component of the antioxidant system can play a significant role in the cellular defense mechanism. Taken together, in view of the strong induction patterns of the *GST* genes of the Arctic green sea urchin *S. droebachiensis*, we propose that this expression profiling could be a significant molecular indicator in a PCB-exposed sample.

GR plays an essential role in maintaining antioxidant capacity by sustaining the reduced status of glutathione (GSH). SOD is an important antioxidant enzyme that catalyzes the conversion of superoxide to oxygen and hydrogen peroxide in a molecular antioxidant defense system. Based on our results, we suggest that PCB mixtures can be a potential modulator of antioxidant defense systems in this species. To date, there have been several reports that exposure to PCBs may induce oxidative stress in a cell or an animal via interaction with the AhR and activation of the cytochrome P4501A (CYP1A) subfamily, resulting in the generation of excess levels of ROS (Morehouse et al. 1984; Oakley et al. 1996; Slim et al. 2000; Schlezinger and Stegeman 2001; Hennig et al. 2002; Schlezinger et al. 2006). Previously, 3,3′,4,4′-tetrachlorobiphenyl treatment induced GR activities in different tissues of rainbow trout (Otto and Moon 1995). Blom and Förlin (1998) reconfirmed that long-term exposure to PCB mixtures induced GR activities in the liver of juvenile rainbow trout. The activities of the cytosolic SOD were significantly higher in the kidneys of the brown bullhead (*Ameiurus nebulosus*) from a PCB-polluted river compared with those from non-polluted sites (Otto and Moon 1996). The waterborne exposure of 0.1 and 10 ppb of PCB mixture (Aroclor 1254) significantly induced the mRNA expression of both SOD genes for 48 h in the digestive gland and the gill tissues of the Antarctic bivalve *Laternula elliptica* (Park et al. 2009b). In addition, a time-course study in response to PCB mixture (Aroclor 1254) treatment revealed a significant increase in the *Cu/ZnSOD* transcript for 24 h in the gill of the disk abalone, *Haliotis discus discus* (De Zoysa et al. 2009). Therefore, the transcriptional expression of oxidative stress-related genes observed in the PCB mixture-exposed *S. droebachiensis* would be suggestive of the oxidative stress-inducing potential of PCB and its metabolites. Regarding the insignificant expression of the *CAT* gene, most laboratory studies have observed equivocal findings in diverse fish exposed to PCBs (reviewed by van der Oost et al. 2003). Since both the induction and suppression of the *CAT* gene have been observed in environmental pollutants, their expressions would be not a valid biomarker for environmental risk assessment (reviewed by van der Oost et al. 2003). Likewise, the *S. droebachiensis CAT* gene would not be expected to be a strong biomarker for PCB in this exposure condition, although experimental replicates were done to confirm its biomarker potential.

Heat shock proteins (*hsps*) are intracellular stress-related proteins that respond to environmental stresses such as temperature, toxins, hypoxia, starvation, magnetic fields, chemical exposure, infection, inflammation, and tumorigenesis (Feder and Hofmann 1999). They also play an important role in protein interactions and folding as a molecular chaperone (Fink 1999). Regarding the transcriptional change in *S. droebachiensis hsps*, the mRNA and protein of the *hsp70* gene were highly induced by PCB 118 exposure in the marine sponge, *Geodia cydonium* (Wiens et al. 1998). In the case of the *hsp90* gene, the level of hsp90 protein was strongly increased after exposure to PCB 118 in the octocoral *Dendronephthya klunzingeri* (Wiens et al. 2000). Regarding the insignificant expression of the *hsc70* gene, the cognate forms of hsp are known to be constitutively expressed compared to its inducible forms, and they are not induced by other stressors including heat exposure. In addition, a PCB mixture (Aroclor 1254) significantly reduced the mRNA of *hsc70* in zebrafish embryos (Kreiling et al. 2007), suggesting that the hsp cognate form has diverse roles beyond its function as a molecular chaperone. Taken together, we suggest that *S. droebachiensis hsps* play a protective role against exogenous and endogenous stressors.

In conclusion, we obtained ESTs from the Arctic green sea urchin *S. droebachiensis* and analyzed the transcriptional expressions of several antioxidant- and cell stress-

related genes upon exposure to a PCB mixture (Aroclor 1254) for 48 h as a preliminary application of the Arctic green sea urchin gene/protein information for environmental biomonitoring. Although we were not able to analyze different time courses due to the difficult sampling of the Arctic green sea urchin, this study revealed the potential usefulness of the Arctic green sea urchin for environmental monitoring of the polar region. In this report, our findings suggest that the detoxification pathway-related genes will provide an early signal of the modulatory effects of such defense systems upon PCB exposure. The ESTs of the Arctic green sea urchin *S. droebachiensis* can have a wide range of applications such as in the RNA sequencing with global transcriptional expression profiling of different environmental conditions of the polar regions.

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