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7A Genomic approaches to coral reef biology

Transcriptomic signature in soft coral exposed to abiotic stresses

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Abstract. The isolation of genes responsive to toxic chemical stress in soft coral (*Scleronephthya gracillimum*) was described. Soft coral colonies were exposed to persistent organic pollutant, benzo(a) pyrene. Gene candidates whose transcript levels changed in response to chemical stress were identified by cDNA library construction. Twenty-five candidate genes were identified from benzo[a]pyrene stress exposed group, which are associated with cell cycle, cell signaling, transcription, translation, protein metabolism, and other cellular functions. The expected function of each gene was described. Among these candidates, the expressional changes of seven genes (protein disulfide isomerase, calreticulin, ferritin, proteasome beta 3 subunit, ribosomal protein L6, hydroxysteroid dehydrogenase, collagen type XXV) were confirmed by real-time quantitative PCR (qRT-PCR). The results suggested that the isolated and identified differentially expressed genes have a potential to identify environmental stressors in environmental changes and could act as molecular biomarkers for biological responses against environmental changes.

Key words: *Scleronephthya gracillimum*, Soft coral, Environmental stress, Transcription.

Introduction

The coral reefs, including soft coral communities, are known to be World's most valuable ecosystems in terms of ecological, economic and cultural capital but are in serious decline mainly due to the human-associated activities. Although the importance of the species has been continuously emphasized and management efforts have been successful locally, the worldwide decline of coral populations due to pollution, disease, and climate change is reaching a crisis. Over the last 30 years, coral reef assessment has provided an extensive description of certain responses at population and community levels in terms of coral cover, diversity and population dynamics of other reef species. However, with only these descriptive approaches for assessment are incapable of identifying the causes of deterioration of coral reef ecosystems. Most of physiological measurements do not identify the stressors or the underlying molecular mechanisms controlling a response. Changes in gene expression and protein production are key elements of the stress response and usually occur before physiological damage is evident. Thus, diagnosis and quantification of the impact of stressors on corals can be possible by using the genes whose expression would turn on or off under a specific type of environmental change.

The soft coral, *Scleronephthya gracillimum* (Fig.1, Alcyonacea, Octocorallia, Anthozoa, Cnidaria), is found predominantly at depths of 15-40 m, in the subtropical ocean regions surrounding Jeju Island, Korea. This species contribute to the species diversity of this area and offer a wide variety of habitats for other benthic marine animals, and facilitate the survival and maintenance of this unique and highly diverse biological community.

The representative environmental stressors in marine ecosystem are anthropogenic contamination such as sewage including persistent organic pollutants and a variety of toxic chemicals from land runoff. Benzo[a]pyrene (BaP) is one of the polycyclic aromatic hydrocarbons (PAHs) and a representative marine ecotoxicant. It has been well reported its bioaccumulative potential in many organisms (Warshawsky, 1999) resulting in DNA damage, endocrine disruption, and reproductive disturbance. In this study, we described the strategy on extensive isolating and identifying both physical and chemical

responsive genes by subtractive cDNA library construction in *S. gracillimum*, and their potential usage as biomarkers to assess the health status of local marine ecosystem.

Material and Methods

Soft Coral and Environmental Stressors Exposure
The *S. gracillimum* soft coral colonies were collected at water depths of approximately 15-25 m near Seogwipo, Jeju Island, Korea, using standard scuba techniques. After transport to the aquatic facility in the laboratory, the specimens were allowed to acclimate for 5 days in 23°C filtered seawater with a salinity of 35 ppt at a light: dark cycle of 14:10 hr. After this acclimation period, control group was kept in 23°C seawater. A BaP-exposed group was assigned to seawater including 100 ppb BaP (dissolved in 0.1% DMSO) (Sigma) for 48 hr and the remaining group was kept in seawater including 0.1% DMSO and used as a control. The colonies were gone into the next step of subtractive cDNA library construction.

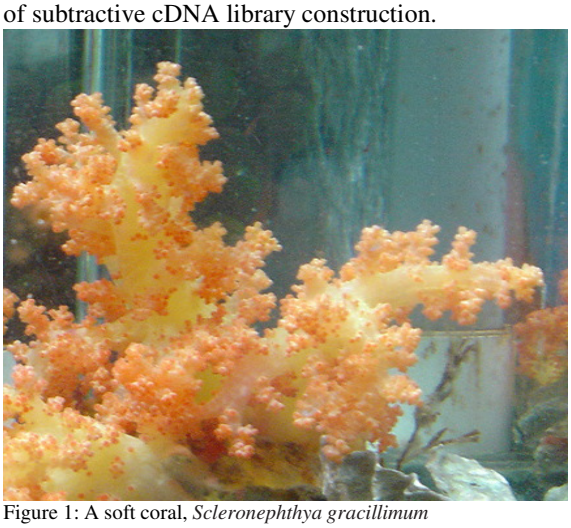

Figure 1: A soft coral, *Scleronephthya gracillimum*

The total RNA was extracted by following the method to be optimized for *S. gracillimum* (Woo et al. 2005). In brief, the soft coral polyp tissues were mortar-pulverized in liquid nitrogen. The polyp powder was then homogenized in 700 µl of lysis solution [35 mM EDTA, 0.7 M LiCl, 7% SDS, 200 mM Tris-Cl (pH 9.0)], and RNA was extracted with 700 µl of water-saturated phenol. One-third-volume of 8 M LiCl was added to the retained aqueous phase, and this was maintained at 4°C for 2 hr. The RNA was precipitated after centrifugation at 14,000 rpm for 30 min and the precipitate was resuspended in 300 µl of DEPC-treated water. The RNA was re-precipitated with a 1/10 volume of 3 M sodium acetate (pH 5.2) and the same volume of isopropanol. The precipitated RNA was rinsed with 70% ethanol (diluted in DEPC-treated water), and dissolved in an appropriate volume of DEPC-treated water (30-40 µl). RNA samples were run on formaldehyde gel in order to verify RNA integrity.

Subtractive cDNA Library Construction
We constructed two subtractive cDNA libraries to identify the differentially expressed genes responding to thermal stress and the chemical contaminant such as BaP. RNA was extracted from soft coral polyp tissues of control and experimental groups. Each subtractive cDNA library was constructed by using PCR-select cDNA subtraction kit (BD Biosciences, San Jose, CA) following the manufacturer's direction. Sequencing of positive clones was carried out with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Changes in the expression of seven genes among the differentially expressed genes were quantified using real-time quantitative RT-PCR analysis, performed in triplicate in 384-well plates using an Applied Biosystems Prism 7900 Sequence Detection System (Applied Biosystems, USA). The β-actin gene was used as an internal control.

Results and Discussion

Among the 700 clones which were randomly sequenced in subtractive cDNA library constructed from stress exposed colonies, we obtained the authentic cDNA clones whose expressions were up- or down-regulated by BaP exposure. Eighteen genes were up-regulated significantly by BaP exposure and seven genes were down-regulated (p<0.05). Potential functions of the genes were listed in Table 1. The GenBank/EMBL/DDBJ accession number of each gene showing highest homology to isolated cDNA clones after using Blastx algorithm of the NCBI server was indicated.

As the initial stage of environmental stress responsive gene isolation in *S. gracillimum*, the reliable candidate genes from organic pollutant exposed colonies were sequenced and searched their homology by blast algorithm in GenBank. We described the expected functions for most of gene candidates in the following sections.

BaP Stress Specific Gene Candidates
G1/S-specific cyclin E1 is essential for the control of the cell cycle at the G1/S transition. GDP dissociation inhibitors are proteins that regulate the GDP-GTP exchange reaction of members of the rab family. Notch protein is transcriptional regulator playing a central role in Notch signaling. The signaling pathway involved in cell-cell communications regulates a broad spectrum of cell fate determinations. NK-4 encodes a homeodomain transcription factor which is

required for development of the dorsal mesoderm and its derivatives in the *Drosophila* embryo (Lee et al. 1997). Hypoxia-inducible factor 1 (HIF1) is a transcription factor that regulates the expression of genes associated with adaptation to the reduced oxygen pressure (Saramaki et al., 2001).

A cathepsin is a member of protease family, which is believed to participate in intracellular degradation and turnover of proteins. It also has been implicated in tumor invasion and metastasis. Focal adhesion kinase (FAK) regulates the cancer cell adhesion and invasion into extracellular matrix (ECM). In addition, phosphorylation of FAK correlates with the increase of cell motility and invasion (Sawai et al., 2005). F-box proteins regulate diverse cellular processes including cell cycle transition, transcriptional regulation and signal transduction (Kuroda et al., 2002). Ras-related protein induces morphological reversion of a transformed cell line. Ras is known to be an oncogene.

The Poly A binding protein (PABP) binds to the 3'-poly(A) tail of mRNA found on most eukaryotic mRNAs and together with the poly(A) tail has been implicated in governing the stability and the translation of mRNA (Gorlach and Dreyfuss, 1994).

	Genes
Up	Protein disulfide isomerase
	Polyglutamine binding protein variant 4 (PQBP1 gene)
	cathepsin
	Calreticulin
	Ferritin
	GTP binding protein
	Poly A binding protein (PABP)
	Eukaryotic translation initiation factor 4
	Proteasome beta 3 subunit
	ATP synthase subunit 8
	G1/S-specific cyclin E1
	HSP70
	HSP90
	Histone H2A, Histone H2A variant
	Bromodomain adjacent to zinc finger domain
	Ferritin
	40S ribosomal protein S23
	Ribosomal protein S8
Do	Ribosomal protein L6
wn	Calcium dependent mitochondrial carrier protein
	Fructose 1,6-bisphosphatase Poly (ADP-ribose) polymerase 4
	Hydroxysteroid dehydrogenase
	Collagen, type XXV
	ATP carrier protein precursor

Table 1. Induction and repression of gene expression by BaP exposure in *S. gracillimum*.

Heat shock protein 90 (HSP90) is a cellular chaperone protein required for the activation of several eukaryotic protein kinases including the cyclin-dependent kinase CDK4. Cytochrome P450 is a family of powerful detox enzymes. UV excision repair protein RAD23 encodes a protein acting in nucleotide excision repair (NER) of UV-damaged DNA (Prakash and Prakash, 2000).

G1 to S phase transition factor and GTP binding protein are involved in regulation of cell growth. Two kinds of protein kinase related genes, activated protein kinase C receptor and serine/threonine protein kinase, were isolated. Eukaryotic protein kinases are enzymes belong to a very extensive protein family which share a conserved catalytic core common with both serine/threonine and tyrosine protein kinases.

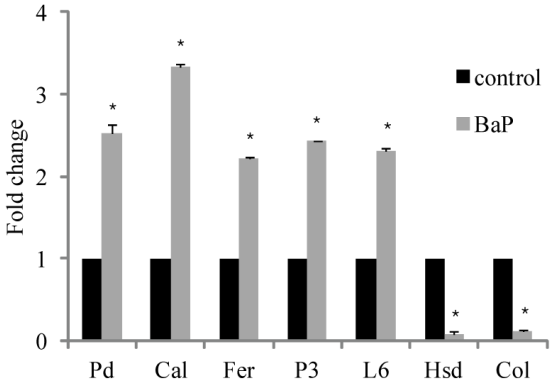

Figure 2. Quantification by real-time RT-PCR of the expression changes of the 7 selected genes. Y axis means values of fold change. Pd, protein disulfide isomerase; Cal, calreticulin; Fer, ferritin; P3, Proteasome beta 3 subunit; L6, ribosomal protein L6; Hsd, Hydroxysteroid dehydrogenase; Col, collagen, type XXV. The β-actin gene was used as an internal control. Each histogram represents the mean ± S.D. (n = 3). *Significantly different from non-exposed control group ($p < 0.05$).

Three kinds of histone homologues, Histone H2A, Histone H2A variant, and Histone H3.2, were found in subtractive cDNA library. Histones are the chief proteins of chromatin. They act as spools around which DNA winds and they play a role in gene regulation.

We also obtained five kinds of ribosomal proteins. One of the genes, ribosomal protein L6 genes were evaluated its expression by real-time PCR (Fig.2). The gene expression was upregulated approximately 2.4-fold comparing with non-exposed control group. Ribosomes are the particles that catalyze mRNA-directed protein synthesis in all organisms. Many of ribosomal proteins, particularly those of the large subunit, are composed of a globular, surfaced-exposed domain with long finger-like projections that extend into the rRNA core to stabilize its structure. The HSP70 family is a set of highly conserved proteins that are induced by a variety of biological

stresses, including heat stress.

ADP, ATP carrier protein precursor catalyzes the exchange of ADP and ATP across the mitochondrial inner membrane. These are transmembrane proteins function in the transport of a wide variety of substrates across extra- and intracellular membranes, including metabolic products, lipids and sterols, and drugs (Higgins, 1992). Tyrosine 3-monooxygenase is an adapter protein implicated in the regulation of both general and specialized signaling pathway. cAMP responsive element modulator (CREM) is one of the nuclear factors involved in the regulation of gene expression by cAMP and has an important role in spermatogenesis. Induced cAMP early repressor has been proposed to function as a tumor or cell proliferation suppressor (Thornberg, 2001).

Figure 2 showed the expressional changes of seven genes confirmed by qRT-PCR. The transcriptions of protein disulfide isomerase, calreticulin, ferritin, proteasome beta 3 subunit, and ribosomal protein L6 increased over 2-fold and the expressions of hydroxysteroid dehydrogenase and collagen type XXV decreased strongly more than 7-fold comparing with non-exposed control group. BaP was known to induce oxidative stress. In this result, the seven genes related to oxidative stress showed the transcriptional changes clearly and it provided the possibility that gene expression changes could be used as a signature induce by chemical exposure stress.

Consequently our results showed that exposure to the organic pollutant like as BaP could modulate the transcription of various genes in *S. gracillimum* and the combination of dynamic gene expression data with cellular effects provided information on the risks of persistent organic pollutant. Each candidate genes could be suggested as biomarkers for assessing the health condition of a soft coral, *S. gracillimum* and have a potential to identify environmental stressors. They could act as molecular indicators for biological responses against environmental changes and the health condition of soft corals may reflect the health condition of entire soft coral community.

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References

Gorlach M, Burd CG, Dreyfuss G (1994) The mRNA poly(A)-binding protein: localization, abundance, and RNA-binding specificity. Experimental Cell Research 211:400-407.

Higgins CF (1992) ABC Transporters: From microorganisms to man. Annual Review of Cell Biology 8:67-113.

Kuroda H, Takahashi N, Shimada H, Seki M, Shinozaki K, Matsui M (2002) Classification and expression analysis of Arabidopsis F-box-containing protein genes. Plant Cell Physiology 43:1073-1085.

Lee YM, Park T, Schulz RA, Kim Y (1997) Twist-mediated activation of the NK-4 homeobox gene in the visceral mesoderm of Drosophila requires two distinct clusters of E-box regulatory elements. Journal of Biological Chemistry 272:17531-17541.

Prakash S, Prakash L (2000) Nucleotide excision repair in yeast. Mutation Research 451:13-24.

Saramaki OR, Savinainen KJ, Nupponen NN, Bratt O, Visakorpi T (2001) Amplification of hypoxia-inducible factor 1alpha gene in prostate cancer. Cancer Genetics and Cytogenetics 128:31-34.

Sawai H, Okada Y, Funahashi H, Matsuo Y, Takahashi H, Takeyama H, Manabe T (2005) Activation of focal adhesion kinase enhances the adhesion and invasion of pancreatic cancer cells via extracellular signal-regulated kinase-1/2 signaling pathway activation. Molecular Cancer 4: 37.

Thonberg H, Lindgren EM, Nedergaard J, Cannon B (2001) As the proliferation promoter noradrenaline induces expression of ICER (induced cAMP early repressor) in proliferative brown adipocytes, ICER may not be a universal tumour suppressor. Biochemical Journal 15:169-177.

Warshawsky D (1999) Polycyclic aromatic hydrocarbons in carcinogenesis. Environmental Health Perspectives 107:317-319.

Woo S, Yum S, Yoon M, Kim SH, Lee J, Kim JH, Lee TK (2005) Efficient isolation of intact RNA from the soft coral Scleronephthya gracillimum (Kükenthal) for gene expression analyses. Integrative Biosciences 9:205-209.