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DOCTORAL THESIS

TOXICOGENOMIC APPROACH TO IMPACT
ASSESSMENT OF WHOLE WASTEWATER EFFLUENTS
AND
DEVELOPMENT OF EFFLUENT-RESPONSIVE
BIOMARKER

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ABSTRACT

The reclaimed wastewater has been served as an alternative water source in some countries but uncertain impacts of wastewater residues including vast categories of micropollutants remains as a challenge to increase the public acceptance. Due to its comprehensive and rapid nature, transcriptome analysis such as DNA microarray and quantitative RT-PCR (qPCR) assay, was applied to human hepatocarcinoma cells (HepG2) exposed to the effluents from membrane bioreactors (MBRs), and the activated sludge process (AS), to better understand the whole effluent impacts on humans, and develop genetic markers for such impacts. The effective reclamation processes were further suggested for the selected wastewater effluents based on the toxicity reduction evaluation study using the genetic markers.

The DNA microarray was first applied to MBR and AS effluents without any enrichment processes. In parallel, the conventional bioassays (i.e., cytotoxicity tests and bioluminescence inhibition test), which were well-established for the evaluation of the overall effluent toxicity were also performed for the same samples. The transcriptome analysis identified 2 to 926 differentially expressed genes after exposure to the effluents and the raw wastewater, which were categorized to 0 to 225 biological processes. Among the tested effluents, the MBR operated at a relatively long solid retention time (i.e., 40 days) and small membrane pore size (i.e., 0.03 μm) was suggested to have the least impacts on the HepG2 even at the level comparable to tap water. The observed gene expression responses were in good agreement with the results of cytotoxicity tests, and provided additional molecular mechanistic information on adverse effects occurred in the sub-lethal region.

To select the effluent-responsive genetic markers, dose-response relationship between limited number of genes and effluent concentrations were studied by using MBR and AS effluents concentrated 10-15 times by reverse osmosis (RO). RO concentration successfully achieved sample enrichment with more than 80% recovery of organic content in the effluents. The qPCR assay demonstrated that four out of nine candidate marker genes, which were selected from the DNA microarray data obtained for the concentrated effluents (i.e., 30 mg/L), had clear concentration-dependency, and thereby suggested relevant as effluent marker genes.

Based on the reduction in gene expression levels of marker genes, effectivity of the selected physicochemical treatment processes were investigated for both simulation of actual reclamation system and identification of responsible fraction. The qPCR assay of four marker genes (i.e., AKR1B10, CYP1A1, GCLM, and GPX2) before and after four typical treatments (i.e., aeration, solid phase extraction with C18, chelating, and ion exchange) together with the detail chemical analysis suggested that hydrophobic organic content, which may be forming complex with metals were responsible for a part of gene expression response observed in HepG2 exposed to the effluents. In addition, it was indicated the possibility that the responsible fraction behave together with humic substances or their building blocks falling in a size range from 300 to thousands of Da. Thus, activated carbon adsorption or reverse osmosis were suggested as key processes in reclamation system of the selected MBR and AS effluents.

At the end, the arsenic-modulated gene expressions were investigated from inorganic arsenic exposure (5 nM to 40 μM as arsenic trioxide for 48 hours). The concentration dependent modulation of gene expression (induction of cell cycle genes, suppression of DNA repair genes, induction of cell cycle arrest genes and apoptotic genes) following exposure to arsenic was further supported by acceleration of cell proliferation, ROS generation, and cytotoxicity. These results indicated the potential pro-carcinogenic actions of inorganic arsenic occur in environmentally relevant exposures. Comparison with gene expression profiles between effluents and

model toxicants revealed the uniqueness of the gene expression profiles of the effluents were suggested.

In conclusion, the transcriptome analysis with human HepG2 cells is a powerful tool to rapidly and comprehensively evaluate impacts of whole wastewater effluents. The effluent-responsive genetic markers were also proposed to quantitatively evaluate the treatability of the wastewater effluents. Based on the response of genetic markers, the activated carbon adsorption was suggested as an effective process to remove responsible fraction, and the importance of final polishing with RO was also supported. Since there is still a gap between the gene expression response and the manifestation of toxicities, the gene expression response should be considered as “potential” of toxicity and thereby, further accumulation of DNA microarray data with well-established toxicity data is indispensable for development of this technology. In addition, due to the change of raw wastewater quality, seasonal variations of effluent impacts are to be investigated based on the gene expression response.

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INTRODUCTION

BACKGROUND

The reclaimed wastewater has been served as an alternative water source in some countries, for irrigation water, industrial water, recreational water, and even for indirect and direct potable water (1,2). Although growing water stress, cost and energy constraints have prompted a call for more widespread utilization of wastewater reclamation and reuse practices, uncertain impacts of vast categories of micropollutants (e.g., pesticide, endocrine disruptors, pharmaceuticals) (3,4,5), poorly characterized or even unknown chemicals in wastewater effluents (e.g., degradation products) (6,7), remains as a challenge to increase the public acceptance. Due to such complex nature of wastewater effluents, it is not feasible that chemical-specific analysis covers all the constituents potentially appear in the effluent, and thereby, the importance of whole effluent evaluation has been recognized (8).

Various types of bioassays have been used to evaluate overall impacts of wastewater effluents. The test organisms used in the previous studies include Japanese medaka, *Daphnia magna*, algae, luminescent marine bacteria, yeast, and mammalian cells (9,10,11,12). However, most of the conventional bioassays targeted on the impacts on non-human organisms, and those on humans have not been fully developed for potable or any other type of reuse practice with a contact with human bodies. Furthermore, since a set of bioassay methods targeting on different endpoints are usually adopted to evaluate the overall effluent toxicities, the test procedure tends to be laborious and time-consuming. Thus, a single more rapid, comprehensive, and sensitive approach needs to be developed.

The recent advances in genomics have provided clues to understand the responses of biota to the environmental pollutants and its associated mechanisms of actions (13,14). The DNA microarray can detect the genome-wide gene expression. The patterns of such gene expression responses represent the primary interactions between the environmental contaminants and biota. Some researchers have applied this technology to characterize the toxicity of mixed chemicals or environmental samples such as polyfluorinated and perfluorinated compounds (15), diesel exhaust particles (16), oil contaminated waters (17,18), and wastewaters from factories (19,20). Those studies demonstrated that DNA microarray analysis could detect the impacts of the samples even without prior concentration, in a relatively short-term exposure from several hours to a couple

of days.

In addition, the observed gene expression responses were found to be associated with various biological processes [e.g., dichloromethane-soluble fraction of diesel exhaust particles induced genes associated drug metabolism, oxidative stress response and cell cycle/apoptosis stress response in mouse alveolar epithelial cells (16); Exposure to treated and untreated textile wastewater altered genes related to ion homeostasis, oxidative stress, detoxification, and mutagenicity or carcinogenesis in yeast (20)]. Since there is still a gap between gene expression responses and the manifestation of actual toxicity, accumulation of microarray data along with the well-established bioassay data is necessary.

On the other hand, mitigation of gene expression alteration by any further treatment method is of great importance as a precautional solution until the relationship between the alteration of ceratin gene (group) and actual manifestation of toxicity is fully understod. Thus, treatibility of wastewater effluents are to be investigated based on their gene expression responses. In terms of treatments, the characterization of physicochemical properties of causative fractions provides more useful information than identification of the specific chemicals since lots of unknown compounds with potential toxicities are expected in the wastewater effluents, and the chemical properties are among the information required to select treatment processes and improve operational conditions.

So far, there has been no researches reporting the relationship between certain gene expression responses and the physicochemical properties of the responsible fraction in the complex environmental samples except for Omura et al. (2009) (16), who revealed that n-hexane soluble fraction and n-hexane insoluble fractions of diesel exhaust particles regulated characteristic genes which respond to chemical properties of each fraction in rat alveolar epithelial cells. Thus, fractionation of complicated environmental samples was demonstrated effective to relate a certain gene expression responses to chemical properties of causative agents.

Toxicity Identification Evaluation (TIE) studies, whose guidelines are available from US-EPA (21,22), provides us with an approach to characterize the cause of observed effluent toxicity, where physical and chemical fractionation with biological toxicity testing allow the characterization and identification of key toxicants in various matrices, such as industrial wastewater, sediment, and leachate (23,24,25,26). Key toxicants identified can be reduced or eliminated by correcting treatment deficiencies or applying specific treatments.

OBJECTIVES

The present study was carried out to reveal impacts of whole wastewater effluents based on gene expression response of cultured human cells, and to develop effluent-responsive biomarker. The achivement in this study is expected to altimately provide single rapid, sensitive and more comprehensive evaluation method of wastewater effluent impacts on humans for increased reliability and efficiency of wastewater reclamation systems. For this objective, the specific aims were set as:

1. to apply DNA microarray-based transcriptome analysis to evaluation of whole wastewater effluents on HepG2
2. to develop marker genes which respond to impacts of wastewater effluents
3. to characterize major contributors to wastewater effluent impacts
4. to better interpret wastewater effluent impacts based on gene expression profiles of model chemicals

OUTLINE of DISSERTATION

This thesis is composed of 7 chapters which include original articles and unpublished work.

Chapter 1 provides a general background of water reclamation practices and bionalytical tools to assess the overall toxicity

of environmental samples including toxicogenomic approaches.

Chapter 2 provides a literature review on the past findings of the overall toxicity of aqueous samples, focusing on the used methods and the effectiveness of the water treatment technologies for the toxicity removal.

In Chapter 3, DNA microarray analysis was applied to impact assessment of whole wastewater effluents from membrane bioreactors (MBRs) and activated sludge process (AS), on the biological processes of human hepatoma HepG2 cells. The conventional bioassays (i.e., cytotoxicity tests and bioluminescence inhibition test) were conducted in parallel since they were well-established evaluation methods for the overall effluent toxicity.

In Chapter 4, DNA microarray was applied to concentrated MBR and AS effluents at sub-lethal concentration (i.e., 30 mg-DOC/L) to select marker genes which respond to wastewater effluent exposure. The selected candidate marker genes were examined for their dose-response relationship.

In Chapter 5, physicochemical properties of biologically active fraction of the effluents were further investigated in various fractionated samples (by size, and chemical properties) using the selected effluent-responsive marker genes.

In Chapter 6, the change in the modes of toxic actions of a model toxicant, arsenic trioxide at the concentrations from environmentally relevant concentrations to apparently toxic concentration, was investigated by DNA microarray analysis.

Finally, Chapter 7 presents the main conclusions extracted from the work presented in this dissertation and a few recommendations for future work.

REFERENCES

- (1) Asano, T., Burton, F. L., Leverenz, H., Tsuchihashi, R., Tchobanoglous, G. Water Reuse: Issues, Technologies, and Applications; McGraw-Hill: New York, U.S., 2007
- (2) Grant, S. B.; Saphores, J.; Feldman, D. L.; Hamilton, A. J.; Fletcher, T. D.; Cook, P. L. M.; Stewardson, M.; Saners, B. F.; Levin, L. A.; Ambrose, R. F.; Deletic, A.; Brown, R.; Jiang, S. C.; Rosso, D.; Cooper, W. J.; Marusic, I., Taking the “Waste” out of “wastewater” for human water security and ecosystem sustainability. *Science* 2012, 337 (6095), 681-686.
- (3) Watkinson, A. J.; Murby, E. J.; Costanzo, S. D., Removal of antibiotics in conventional and advanced wastewater treatment: Implications for environmental discharge and wastewater recycling. *Water Res* 2007, 41 (18), 4164-4176.
- (4) Kimura, K.; Hara, H.; Watanabe, Y., Elimination of selected acidic pharmaceuticals from municipal wastewater by an activated sludge system and membrane bioreactors. *Environ Sci Technol* 2007, 41 (10), 3708-3714.
- (5) Hollender, J.; Zimmermann, S. G.; Koepke, S.; Krauss, M.; McArdell, C. S.; Ort, C.; Singer, H.; von Gunten, U.; Siegrist, H., Elimination of Organic Micropollutants in a Municipal Wastewater Treatment Plant Upgraded with a Full-Scale Post-Ozonation Followed by Sand Filtration. *Environ Sci Technol* 2009, 43 (20), 7862-7869.
- (6) Escher, B. I.; Fenner, K., Recent Advances in Environmental Risk Assessment of Transformation Products. *Environ Sci & Technol* 2011, 45 (9), 3835-3847.
- (7) Feron, V. J.; Cassee, F. R.; Groten, J. P.; van Vliet, P. W.; van Zorge, J. A., International issues on human health effects of exposure to chemical mixtures. *Environ Health Perspect* 2002, 110, 893-899.
- (8) Brack, W., Effect-directed analysis: a promising tool for the identification of organic toxicants in complex mixtures? *Anal Bioanal Chem* 2003, 377 (3), 397-407.
- (9) Cao, N.; Yang, M.; Zhang, Y.; Hu, J. Y.; Ike, M.; Hirotsuji, J.; Matsui, H.; Inoue, D.; Sei, K., Evaluation of wastewater reclamation technologies based on in vitro and in vivo bioassays. *Sci Total Environ* 2009, 407 (5), 1588-1597.
- (10) Kontana, A.; Papadimitriou, C. A.; Samaras, P.; Zdragas, A.; Yiangou, M., Bioassays and biomarkers for ecotoxicological assessment of reclaimed municipal wastewater. *Water Sci Technol* 2008, 57 (6), 947-953.
- (11) Petala, M.; Samaras, P.; Kungolos, A.; Zouboulis, A.; Papadopoulos, A.; Sakellariopoulos, G. P., The effect of coagulation on the toxicity and mutagenicity of reclaimed municipal effluents. *Chemosphere* 2006, 65 (6), 1007-1018.
- (12) Macova, M.; Toze, S.; Hodgers, L.; Mueller, J. F.; Bartkow, M.; Escher, B. I., Bioanalytical tools for the evaluation of organic micropollutants during sewage treatment, water recycling and drinking water generation. *Water Res* 2011, 45 (14), 4238-4247.
- (13) Mahadevan, B.; Snyder, R. D.; Waters, M. D.; Benz, R. D.; Kemper, R. A.; Tice, R. R.; Richard, A. M., Genetic Toxicology in the 21st Century: Reflections and Future Directions. *Environ Mol Mutagen* 2011, 52 (5), 339-354.
- (14) Aardema, M. J.; MacGregor, J. T., Toxicology and genetic toxicology in the new era of “toxicogenomics”: impact of “-omics” technologies. *Mutat Res-Fund Mol M* 2002, 499 (1), 13-25.
- (15) Wei, Y.; Shi, X.; Zhang, H.; Wang, J.; Zhou, B.; Dai, J., Combined effects of polyfluorinated and perfluorinated compounds on primary cultured hepatocytes from rare minnow (*Gobiocypris rarus*) using toxicogenomic analysis. *Aquat Toxicol* 2009, 95(1): 27-36.
- (16) Omura, S.; Koike, E.; Kobayashi, T., Microarray analysis of gene expression in rat alveolar epithelial cells exposed to fractionated organic extracts of diesel exhaust particles. *Toxicology* 2009, 262(1): 65-72.
- (17) Lie, K. K.; Meier, S.; Olsvik, P. A., Effects of environmental relevant doses of pollutants from offshore oil production on Atlantic cod (*Gadus morhua*). *Comp Biochem Physiol C* 2009, 150(2), 141-149.
- (18) Olsvik, P. A.; Lie, K. K.; Stavrum, A. K.; Meier, S., Gene-expression profiling in gill and liver of zebrafish exposed

- to produced water. *Int J Environ Anal Chem* 2007, 87(3), 195-210.
- (19) Moens, L. N.; Smolders, R.; van der Ven, K.; van Remortel, P; Del-Favero, J.; De Coen, W. M., Effluent impact assessment using micro array-based analysis in common carp: A systems toxicology approach. *Chemosphere* 2007, 67(11), 2293-2304.
 - (20) Kim, H. J.; Rakwal, R.; Shibato, J.; Iwahashi, H.; Choi, J. S.; Kim, D. H., Effect of textile wastewaters on *Saccharomyces cerevisiae* using DNA microarray as a tool for genome-wide transcriptomics analysis. *Water Res* 2006, 40(9), 1773-1782.
 - (21) United States Environmental Protection Agency (US-EPA). *Methods for Aquatic Toxicity Identification Evaluations - Phase I Toxicity Characterization Procedures*, 2nd, ed.; Office of Research and Development, Washington, DC, 1991
 - (22) United States Environmental Protection Agency (US-EPA). *Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants*; Office of Research and Development, Washington, DC, 1999
 - (23) Erten-Unal, M.; Gelderloos, A. B.; Hughes, J. S., A toxicity reduction evaluation for an oily waste treatment plant exhibiting episodic effluent toxicity. *Sci Tot Environ* 1998, 218, 141-152.
 - (24) Jo, H.; Park, E.; Cho, K.; Kim, E.; Jung, J., Toxicity identification and reduction of wastewaters from a pigment manufacturing factory 2008, *Chemosphere*, 70, 949-957.
 - (25) Krantzberg, G.; Boyd, D., The biological signifiance of contaminants in sediments from hamilton harbour, Lake Ontario. *Environ Toxicol Chem* 1992, 11(11), 1527-1540.
 - (26) Jemec, A.; Tisler, T.; Zgajnar-Gotvajn, A., Assessment of landfill leachate toxicity reduction after biological treatment. *Arch Environ Contam Toxicol* 2012, 62(2), 210-21.

2

LITERATURE REVIEW

Over the several decades, various types of bioassays have been used to investigate the toxicity of water samples for protection of aquatic environment and human health. Some applied bioassays to artificial mixtures to simulate additive, synergistic, or antagonistic effects of specific chemicals of their interest in the samples, while others to actual wastewaters from households and industries. In this chapter, giving more focus on the latter cases, the previous findings in the application of bioassays to the overall toxicity evaluation of water samples were summarized.

Sample type

The aqueous mixtures examined in the previous studies are categorized in **Table 2-1**. In a controlled system, artificial mixtures of metals and emerging compounds have been investigated. The metal mixtures of interest were the combination of 6 metals (i.e., Hg, Cd, Cu, Zn, Ni and Pb) (1) and 3 binary systems (i.e., Cd-As, Cd-Ni, and Ni-Pb) (2,3). Among emerging compounds, organophosphates (insecticides) are tested in 6 and 15 compound system (4,5), while diquat and fomsafen (herbisides) are tested in a binary mixture (6). Endocrine disrupting compounds, pharmaceuticals and other trace organic pollutants were also studied (7-10),

Actual environmental samples were examined as well. The studies examined municipal wastewaters had more focus on the removal of the overall toxicity, while those used industrial wastewater and environmental water samples had a larger interest in the evaluation of ecological impact of the specific (potentially) contaminated site. Not only the secondary sewage effluent (11-25) but also the tertiary treated wastewater were investigated for the overall toxicity, the treatment technologies varying from, membrane bioreactors (MBRs) (24), ozonation (14,17,18,21), activated carbon (AC) (20,25) to nanofiltration (NF) (14,21,25), and reverse osmosis (RO) (14).

The tested industrial wastewater included discharges or leachates from oil refinery plant (26-30), tannery factory (31), textile factory (32), electroplating factory (33) and circulated water at an aquaculture site (34) Potentially contaminated environmental samples included river water and groundwater (35-40).

Table 1 Aqueous mixtures examined in the previous studies

Category	Aqueous mixture
Artificial chemical mixtures	Heavy metals (3), Pesticides (3), EDCs (1), Pharmaceuticals (1), PFCs (1), PAHs (1), Non-ionic surfactants (1), etc.
Municipal wastewater with or without treatment	Secondary Effluent (15), SE+O ₃ (4), SE+Coagulation (4), SE+MF/NF/RO (4), MBR (3), SE+AC (2), SE+A ₂ O (2)
Industrial wastewater with or without treatment	Oil (6), Tannery (1), Textile (1), Electroplating (1), Aquaculture (1), Unspecified (1)
Potentially contaminated environmental water	River water (3), Groundwater (1)

*Bracketed number stands for the number of cases.

Endpoints

The methods used for measurement of mixture toxicity are listed in **Table 2-2**. Most of the studies used bioassays, which evaluate the toxicity of novel or unknown chemical based on the response of the living organisms exposed to them, to investigate the overall toxicity of the targeted mixtures. Various *in vivo* bioassays using marine organisms were examined. Among the listed methods, *D. magna* proliferation/immobility assay and algae proliferation test are standardized evaluation method for ecotoxicity.

In vitro assays with their advantages in time-saving and simple procedures were applied to a wide range of water samples. The standardized methods include bioluminescence inhibition assay using *Vibrio fischeri* (general toxicity), Ames test and umu assay using *Salmonella typhimurium* (genotoxicity). Among bioassays for evaluating various impacts of chemicals on humans and ecosystems, those based on cultured mammalian-cells can best predict acute lethal toxicity to humans. *In vitro* bioassay using mouse spleen cell, cell and human hepatocarcinoma cell line (HepG2) were used for river water and secondary sewage treatment.

In recent years, toxicogenomics based on microarray analysis have been increasingly applied in the characterization of the cellular effects of single and chemical exposures. In the assessment of overall toxicity of aqueous samples, the gene expression analysis was used for *D. magna* exposed to metal mixtures, primary cultured hepatocytes of rare minnows exposed to perfluorocarbons, and several types of fishes and marine copepod *Calanus finmarchicus* grown in the oil contaminated waters. So far, there has been no application of the toxicogenomic approach to toxicity assessment of the tertiary treated wastewater.

Table 2 Methods applied for measuring overall toxicity

Category	Methods	Endpoint
<i>In vivo</i> bioassay	Algae	proliferation, survival, growth in size/weight, immobility (<i>D.magna</i>), gene expression, metabolism activity
	Plankton	
	Shrimp/Oyster	
	Marine plant	
	Fish	
<i>In vivo</i> bioassay	Bacteria	<i>proliferation, survival, gene expression, metabolism activity</i>
	Mammalian cell	
	Direct chemical reaction	

Application of DNA microarray to toxicological studies

Moens et al. (2007) (36) investigated the transcriptomic response of common carp exposed to industrial wastewater, using cDNA microarray with 960 carp gene fragments. The time-course analysis revealed that a number of differentially expressed genes remained more or less constant during the 21-day experiment. In addition, there was a significant overlap of differentially expressed genes between the different time points investigated. These genes indicated alterations in the metabolism of both endogenous and exogenous energy resources, including changes in carbohydrate and lipid metabolism, as well as digestive enzyme activity.

In vitro systems, Olsvik et al. (2007) (29) and Kim et al. (2006) (33) applied the DNA microarray to produced water and textile mill effluents (TMEs), respectively. Olsvik et al. found differentially expressed 27 genes and 55 genes in gills and liver tissues of zebrafish exposed to 5% produced water (by-product of oil and gas extraction). The produced water may be the mixture of inorganic salts, heavy metals, production chemicals, hydrocarbons, benzene, polyaromatic hydrocarbons (PAHs). CYP1A was up-regulated to the highest degree in the gill tissue. The protein encoded by CYP1A is a member of the cytochrome P450 superfamily of enzymes, which plays a key role in many xenobiotics metabolism.

To investigate potential toxicity of textile mill effluents (TMEs), Kim et al. examined the genome-wide gene expression profiles of model eukaryote, *Saccharomyces cerevisiae* exposed to TMEs. Among 315 differentially expressed genes, genes related to oxidative stress were still up-regulated in treated effluents. Besides, alteration of genes related to mutagenicity or carcinogenesis were also observed in treated and untreated TMEs. Based on these result, Kim et al. claimed that even after treatment the wastewater posed a certain level of risk to the environment.

Secondary effluent (SE).

The previous studies of secondary observed in sewage effluents are summarized in **Table 2-3**. M. Farre et al. (2001) (11) demonstrated that municipal wastewater received textile and tannery discharges were more toxic than municipal wastewater only, in terms of bioluminescence inhibition of *V.fischeri*. At the same time, toxicity to the algae, human cells and Japanese medaka were observed in the secondary sewage itself. J. Zha and Z. Wang (2005) (25) concluded that secondary treatment was only partly effective to the removal of acute toxicants to Japanese medaka embryo, while H. Narita et al. (2007) (13) detected even higher toxicity of the secondary effluents toward the human neuroblastoma cell lines than the influent and return flows from sludge treatment facility. They considered that by-products during the activated sludge process, suggested as organic matter released from bacteria decay, increased the toxicity. The acute invertebrate toxicity was also observed by N. Cao et al. (2009) (14), who showed the secondary effluent had genotoxicity and weak RAR α sec as well.

Table 3 Overall toxicity of secondary sewage effluent

Study	Methods	Endpoint	Results
M. Farre et al. (2001)	<i>V. fischeri</i>	Bioluminescence inhibition	✓ Samples with high contents of industrial wastes from textile and tannery industries are most toxic ✓ Some treatments were not sufficient and effluent still toxic due to compounds like some phenols and nonylphenol
Y. Liang and M.H. Wong (2000)	1) <i>Chlorella</i> G 2) <i>Gambusia</i>	1) Cell growth 2) Mortality	✓ Only sewage effluent from Yuen Long had contaminants at toxic levels ✓ Total ammonia in water explained >80% variations of toxicity of water water samples to Gambusi
H. Narita et al. (2007)	Human neuroblastoma cell lines (NB-1)	Cell viability	✓ Toxicity of effluent was more intensive than that of influent and return flows from sludge treatment facility in investigated wastewater treatment plant (Organic matter released from activated sludge bacteria during their decay process contributed to increase in toxicity in effluent)
J. Zha and Z. Wang (2005)	Japanese medaka embryo and larval	Morphology, Mortality	✓ Acute toxicity present in sewage water and secondary treatment could remove only part of toxicants
N. Cao et al. (2009)	1) <i>Salmonella typhimurium</i> TA1535/pSK1002 2) <i>Saccharomyces crevisiae</i> Y190 3) <i>D. magna</i> 4) Japanese medaka embryo	1) Bacterial density 2) RAR α activity 3) Mortality 4) Mortality	✓ Secondary effluent have genotoxicity, weak RARα activity, acute invertebrate toxicity as well as remarkably adverse effect on medaka embryos development

SE+ Chlorination/UV disinfection.

The previous studies on the effectiveness of secondary treatment–ozonation system are summarized in **Table 3–4**. L. Using the bioluminescence inhibition assay, both Wang et al. (2007) (9) and D. Wei et al. (2006) (10) reached the similar conclusion that the toxicity of wastewater samples more or less increased during chlorine disinfection. It was possibly due to the formation of toxic disinfection byproduct, expected from the significant correlation of toxicity with chlorine dosage, ammonia and organic carbon concentrations. N. Cao et al. (14) also pointed out the possible relation of chlorinated byproducts using a bioassay battery and at the same time, demonstrated that UV elicited abatement of genotoxicity only at a high dose.

Table 4 Effectiveness of secondary treatment–chlorination/UV coagulation system on toxicity removal

Study	Methods	Endpoint	Results
N. Cao et al. (2009)	Same as above (see Table. 3)		✓ UV elicit abatement of genotoxicity only at a high dose; Ozonation found effective to remove genotoxicity ✓ Increase of genotoxicity and acute invertebrate toxicity observed in chlorinated effluents maybe due to chlorinated byproducts
L. Wang et al. (2007)	<i>Photobacterium phosphoreum</i>	Luminescence inhibition	✓ Toxicity of wastewater samples significantly increased after chlorine disinfection with formation of toxic DBPs; Both ammonia and organic carbon may react with chlorine disinfectant; statistical analyses indicated toxicity formation significantly correlated with chlorine dosage. ammonia and organic carbon concentrations
D. Wei et al. (2006)	<i>V. fischeri</i>	Bioluminescence inhibition	✓ Toxicity decreased along conventional reclamation processes, while it significantly increased during chlorination disinfection; toxicity of residual chlorine in a combined form increased with chlorine dosage (luminescence inhibition linear with logarithm of chlorine dosage); toxicity of free chlorine increased to a peak and decreased with increasing chlorine dosage; UV254 increase toxicity formation of disinfected wastewater while NH₃-N had decreased

SE+ Ozonation.

The previous studies on the effectiveness of secondary treatment-ozonation system are summarized in **Table 2-5**. A battery of 5 bioassays were developed and examined against the toxicity of the tertiary treated secondary sewage effluents by M. Petala et al. (17,18). They exhibited that toxicity increased after ozonation in most case but they are potentially susceptible to the hepatic metabolism. Other than bioassays, simulation was also used to estimate the mixture toxicity of pesticides and pharmaceuticals based on the literature data. In terms of genotoxicity, N. Cao et al. (14) and Y. Ono et al. (21) suggested ozonation as an effective removal technology as long as the ozone dose is less than 4 mg/L.

Table 5 Effectiveness of secondary treatment-ozonation system on toxicity removal

Study	Methods	Endpoint	Results
M. Petala et al. (2008)	1) <i>V. fischeri</i> 2) <i>Salmonera typhimurium</i> TA100 and TA98	1) Bioluminescence inhibition 2) Mutation ratio (enzyme activity)	✓ In most cases, toxicity increased and reached up to 100% after ozonation with 8.0 mgO₃/L for 5 min ✓ Mutagenic activity towards strain TA98 without metabolic activation reduce, when ozon dose and contact time increased, but also increased at low ozone doses and for contact time > 5 min ✓ Mutagenic activity towards strain TA98 with metabolic activation remained about same or reduced, even eliminated after ozonation with 8.0 mgO₃/L for 5 min
M. Petala et al. (2006)	1) <i>V. fischeri</i> 2) <i>D. magna</i> 3) <i>D. pulex</i> 4) <i>Thamnocephalus platyurus</i> 5) <i>Brachionus calyciflorus</i>	1) Bioluminescence inhibition 2) Mortality 3) Mortality 4) Mortality 5) Mortality	✓ <i>D. pulex</i> was the most susceptible organism for evaluation of reclaimed wastewater toxicity ✓ Microtox test was not sensitive for toxicity of coagulated effluents but sensitive toward intermediate ozonation byproducts;
N. Cao et al. (2009)	Same as above (see Table. 3)		✓ Ozonation found effective to remove genotoxicity, RARα agonist activity and invertebrate toxicity but ozonated effluent at higher than 4mg/L would elicit significant decrease of hatching success of medaka embryos
Y. Ono et al. (1996)	<i>Salmonella typhimurium</i> TA1535/pSK1002, Newly developed <i>S. typhimurium</i> NM strains	Enzyme activity	✓ Detected genotoxicity may depend on human feces and urine ✓ Ozonation effective to reduce genotoxic activity (about 0.5-1.0 mgO₃)

SE+ Coagulation.

The previous studies on the effectiveness of secondary treatment-coagulation system are summarized in **Table 2-6**. N. Cao et al. (14) and Y. Ono et al. (21) demonstrated that coagulation with following sandfiltration or UF couldn't reduce genotoxic activity. On the other hand, M. Petala et al. (19) suggested that the aluminum coagulants, which resulted in the extended removal of organic matter, were more effective than ferric chloride on the removal of mutagenic potency.

Table 6 Effectiveness of secondary treatment-coagulation system on toxicity removal

Study	Methods	Endpoint	Results
M. Petala et al. (2006)	Same as above (see Table. 5)		✓ Mutagenic effects on strain TA98 increased during coagulation with ferric chloride ✓ Addition of aluminum coagulants resulted in a decrease of mutagenic potential of secondary effluents due to extended removal of organic matter
N. Cao et al. (2009)	Same as above (see Table. 3)		✓ Coagulation with following sandfiltration and UF showed little effect on genotoxicity, RAR α activity and acute invertebrate toxicity
Y. Ono et al. (1996)	Same as above (see Table. 5)		✓ Genotoxic activity cannot be reduced by UF nor by coagulation/UF

SE+ Activated Carbon (AC).

The previous studies on the effectiveness of secondary treatment-activated carbon system are summarized in **Table 2-7**. Both of two available studies concluded that activated carbon adsorption was one of the most effective treatments for toxicity removal. A. Kontana et al. (2008) (20) reported that all of the tested 4 types of bioassays indicated the reduction of toxicity. However, they observed both spleen cell mitogenic responses and immune response induced even after the treatment. In addition, J. Zha and Z. Wang (25) pointed out that discharge without dilution could still manifest potential ecological risk.

Table 7 Effectiveness of secondary treatment-activated carbon system on toxicity removal

Study	Methods	Endpoint	Results
A. Kontana et al. (2008)	1) <i>V. fischeri</i> 2) <i>D. magna</i> 3) Protozoan <i>Tetrahymena thermophila</i> 4) Mouse spleen cells	1) Bioluminescence inhibition 2) Mortality 3) Growth inhibition 4) ³ H-TdR incorporation, cytokine production	✓ In all cases responses of organisms used in bioassays decreased after application of activated carbon to samples (close to control values) ✓ No significant changes observed in <i>D. magna</i> responses ✓ Both spleen cell mitogenic responses and cytokines as biomarkers proved to be very sensitive tools since immune response induced even after activated carbon
J. Zha and Z. Wang (2005)	Japanese medaka embryo and larval	Morphology, Mortality	✓ Acute toxicity present in sewage water and secondary treatment could remove only part of toxicants; Membrane technologies could not further remove trace toxicants and endocrine disruptors; Activated carbon adsorption and reverse osmosis effective in removing most of toxicants but discharge without dilution could still manifest potential ecological risk

SE+ Membrane Bioreactor (MBRs).

The previous studies on the effectiveness of membrane bioreactors system are summarized in **Table 2-8**. So far, only G. Libralato et al. (24) have reported on the MBR installing UF membrane. They applied the MBR with UF membrane both to commercial and mixed domestic and metal industries wastewaters and have show that the technology was able to provide superior quality effluents based on the physic-chemical analyses.

Table 8 Effectiveness of secondary treatment-MBRs system on toxicity removal

Study	Methods	Endpoint	Results
G. Libralato et al. (2010)	1) <i>V. fixcheri</i> 2) <i>C. gigas</i> 3) <i>M. galloproincialis</i>	1) Bioluminescence inhibition 2) N/A 3) N/A	✓ MBR installing UF membrane applied both to commercial and mixed domestic and metal industries wastewaters have shown to be able to provide superior quality effluents ✓ Some of the very strict regulatory limits were sporadically slightly exceeded

SE+ Nanofiltration (NF)/ Ultrafiltration (UF)/ Reverse Osmosis (RO).

The previous studies on the effectiveness of Nanofiltration, Ultrafiltration or Reverse Osmosis system are summarized in Table 9. Y. Ono et al. (21) and J. Zha and Z. Wang (25) demonstrated that neither NF nor UF could further remove trace toxicants. On the other hand, RO was suggested as the most effective technologies by Cao et al. (14).

Table 2-9 Effectiveness of secondary treatment- MF/UF/RO system on toxicity removal

Study	Methods	Endpoint	Results
Y. Ono et al. (1996)	Same as above (see Table. 5)		✓ Genotoxic activity cannot reduced by UF nor by coagulation/UF ✓ LC applied to divide genotoxic fractions induced by aromatic amines
J. Zha and Z. Wang (2005)	Same as above (see Table. 7)		✓ UF and MF could not further remove trace toxicants and endocrine disruptors ✓ Activated carbon adsorption and reverse osmosis effective in removing most of toxicants but discharge without dilution could still manifest potential ecological risk
N. Cao et al. (2009)	Same as above (see Table. 3)		✓ RO is the most effective technologies to remove most toxicants from the secondary effluent ✓ Coagulation-sand filtration and UF showed little effect on genotoxicity, RAR α activity and acute invertebrate toxicity

REFERENCES

- (1) Verslycke, T.; Vangheluwe, M.; Heijerick, D.; De Schamphelaere, K.; Van Sprang, P.; Janssen, C. R., The toxicity of metal mixtures to the estuarine mysid *Neomysis integer* (Crustacea: Mysidacea) under changing salinity. *Aquat Toxicol* 2003, 64(3), 307-315.
- (2) Vandenbrouck, T.; Soetaert, A.; van der Ven, K.; Blust, R.; De Coen, W., Nickel and binary metal mixture responses in *Daphnia magna*: Molecular fingerprints and (sub)organismal effects. *Aquatic Toxicol* 2009, 92(1), 18-29.
- (3) Liu, J.; Habeebu, S. M.; Waalkes, M. P.; Klaassen, C. D., Chronic combined exposure to cadmium and arsenic exacerbates nephrotoxicity particularly in metallothionein-I/II null mice. *Toxicology* 2000, 247(3), 157-166.
- (4) Chevre, N.; Maillard, E.; Loepfe, C.; Becker-van Slooten, K., Determination of water quality standards for chemical mixtures: Extension of a methodology developed for herbicides to a group of insecticides and a group of pharmaceuticals. *Exotoxicol Environ Saf* 2008, 71(3), 740-748.
- (5) Tahara, M.; Kubota, R.; Nakazawa, H.; Tokunaga, H.; Nishimura, T., Use of cholinesterase activity as an indicator for the effects of combinations of organophosphorus pesticides in water from environmental sources. *Water Res* 2005, 39(20), 5112-5118.
- (6) Gorzerino, C.; Quemeneur, A.; Hillenweck, A.; Baradat, M.; Delous, G.; Ollitrault, M.; Azam, D.; Caquet, T.; Laquadic, L., Effects of diquat and fomesafen applied alone and in combination with a nonylphenol polyethoxylate adjuvant on *Lemna minor* in aquatic indoor microcosms. *Exotoxicol Environ Saf* 2009, 72(3), 802-810.
- (7) Brian, J. V.; Harris, C. A.; Scholze, M.; Backhaus, T.; Booy, P.; Lamoree, M.; Pojana, G.; Jonkers, N.; Runnalls, T.; Bonfa, A.; Marcomini, A.; Sumpter, J. P., Accurate prediction of the response of freshwater fish to mixture of estrogenic chemicals. *Environ Health Perspect* 2005, 113(6), 721-728.
- (8) Cleuvers, M., Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. *Ecotoxicol Environ Saf* 2004, 59(3), 309-315.
- (9) Wang, L.; BZheng, B.; Meng, W., Photo-induced toxicity of four polycyclic aromatic hydrocarbons, singly and in combination, to the marine diatom *Phaeodactylum tricornutum*. *Ecotoxicol Environ Saf* 2008, 72(2), 465-472.
- (10) Wei, Y.; Shi, X.; Zhang, H.; Wang, J.; Zhou, B.; Dai, J., Combined effects of polyfluorinated and perfluorinated compounds on primary cultured hepatocytes from rare minnow (*Gobiocypris rarus*) using toxicogenomic analysis. *Aquat Toxicol* 2009, 95(1), 27-36.
- (11) Farre, M.; Garcia, M.; Tirapu, L.; Ginebreda, A.; Barcelo, D., Wastewater toxicity screening of non-ionic surfactants by Toxalert and Microtox bioluminescence inhibition assays. *Anal Chim Acta* 2001, 427(2), 181-189.
- (12) Liang, Y.; Wong, M. H., Reclamation of wastewater for polyculture of freshwater fish: Bioassays using *Chlorella* and *Gambusia*. *Arch Environ Contam Toxicol*, 39(4), 506-514.
- (13) Narita, H.; Abe, J.; Funamizu, N.; Takakuwa, T.; Kunimoto, M., Toxicity assessment of treated wastewater using cultured human cell lines. *Environ Monit Assess* 2007, 129(1-3), 71-77.
- (14) Cao, N.; Yang, M.; Zhang, Y.; Hu, J.; Ike, M.; Hirotsuji, J.; Matsui, H.; Inoue, D.; Sei, K., Evaluation of wastewater reclamation technologies based on in vitro and in vivo bioassays. *Sci Total Environ* 2009, 407(5), 1588-1587.
- (15) Macova, M.; Escher, B. I.; Reungoat, J.; Carswell, S.; Chue, K. L.; Keller, J.; Mueller, J. F., Monitoring the biological activity of micropollutants during advanced wastewater treatment with ozonation and activated carbon filtration. *Water Res* 2010, 44(2), 477-492.
- (16) Reungoat, J.; Macova, M.; Escher, B. I.; Carswell, S.; Mueller, J. F.; Keller, J., Removal of micropollutants and reduction of biological activity in a full scale reclamation plant using ozonation and activated carbon filtration.

- Water Res 2010, 44(2), 625-637.
- (17) Petala, M.; Samaras, P.; Zouboulis, A.; Kungolos, A.; Sakellaropoulos, G. P., Influence of ozonation on the in vitro mutagenic and toxic potential of secondary effluents. *Water Res* 2008, 42(20), 4929-4940.
 - (18) Petala, M.; Samaras, P.; Zouboulis, A.; Kungolos, A.; Sakellaropoulos, G., Ecotoxicological properties of wastewater treated using tertiary methods. *Environ Toxicol* 2006, 21(4), 417-427.
 - (19) Petala, M.; Samaras, P.; Kungolos, A.; Zouboulis, A.; Papadopoulos, A.; Sakellaropoulos, G. P., The effect of coagulation on the toxicity and mutagenicity of reclaimed municipal effluents. *Chemosphere* 2006, 65(6), 1007-1018.
 - (20) Kontana, A.; Papadimitriou, C. A.; Samaras, P.; Zdraqas, A.; Yianqou, M., Bioassays and biomarkers for ecotoxicological assessment of reclaimed municipal wastewater. *Water Sci Technol* 2008, 57(6), 947-953.
 - (21) Ono, Y.; Somiya, I.; Kawaguchi, T.; Mohri, S., Evaluation of toxic substances in effluents from a wastewater treatment plant. *Water Reuse* 1996, 106(1-3), 255-261.
 - (22) Wang, L.; Wei, D.; Wei, J.; Hu, H., Screening and estimating of toxicity formation with photobacterium bioassay during chlorine disinfection of wastewater. *J Hazard Mater* 2007, 141(1), 289-294.
 - (23) Wei, D.; Wang, L.; Wei, J.; Hu, H. Y., Toxicity screening and evaluating in chlorination disinfection of wastewater reclamation processes. *Water Sci Technol* 2006, 53(9), 329-346.
 - (24) Libralato, G.; Volpi Ghirardini, A.; Avezzu, F., Toxicity removal efficiency of decentralised sequencing batch reactor and ultra-filtration membrane bioreactors. *Water Res* 2010, 44(15), 4437-4450.
 - (25) Zha, J.; Wang, Z., Assessing technological feasibility for wastewater reclamation based on early life stage toxicity of Japanese medaka (*Oryzias latipes*). *Agric Exosyst Environ* 2005, 107(2-3), 187-198.
 - (26) Hansen, B. H.; Altin, D.; Nordtug, T.; Olsen, A. J., Suppression subtractive hybridization library prepared from the copepod *Calanus finmarchicus* exposed to a sublethal mixture of environmental stressors. *Comp Biochem Physiol Part D Genomics Proteomics* 2007, 2(3), 250-256.
 - (27) Paixao, J. F.; Nascimento, I. A.; Pereira, S. A.; Leite, M. B.; Carvalho, G. C.; Silveira, J. S. Jr.; Reboucas, M.; Matias, G. R.; Rodrigues, I. L., Estimating the gasoline components and formulations toxicity to microalgae (*Tetraselmis chuii*) and oyster (*Crassostrea rhizophorae*) embryos; An approach to minimize environmental pollution risk. *Environ Res* 2007, 103(3), 365-374.
 - (28) Erten-Unal, M.; Gelderloos, A. B.; Hughes, J. S., A toxicity reduction evaluation for an oily waste treatment plant exhibiting episodic effluent toxicity. *Sci Total Environ* 1998, 218(2-3), 141-152.
 - (29) Olsvik, P. A.; Lie, K. K.; Stavrum, A.; Mier, S., Gene-expression profiling in gill and liver of zebrafish exposed to produced water. *Int J Environ Anal Chem* 2007, 87(3), 195-210.
 - (30) Hook, S. E.; Lampi, M. A.; Febbo, E. J.; Ward, J. A.; Parkerton, T. F., Temporal patterns in the transcriptomic response of rainbow trout, *Oncorhynchus mykiss*, to crude oil. *Aquat Toxicol* 2010, 99(3), 320-329.
 - (31) Siwik, P. L.; Meer, T. V.; MacKinnon, M. D.; Paszkowski, C. A., Growth of fathead minnows in oil-sand-processed wastewater in laboratory and field. *Environ Toxicol Chem* 2000, 19(7), 1837-1845.
 - (32) Schrank, S. G.; Bieling, U.; Jose, H. J.; Moreira, R. F.; Schroder, H. F., Generation of endocrine disruptor compounds during ozone treatment of tannery wastewater confirmed by biological effect analysis and substance specific analysis. *Water Sci Technol* 2009, 59(1), 31-8.
 - (33) Kim, H.; Rakwal, R.; Shibato, J.; Iwahashi, H.; Choi, J. S.; Kim, D. H., Effect of textile wastewaters on *Saccharomyces cerevisiae* using DNA microarray as a tool for genome-wide transcriptomics analysis. *Water Res* 2006, 40(9), 1773-1782.
 - (34) Horvat, T.; Vidakovic-Cifrek, Z.; Orescanin, V.; Tkalec, M.; Pevalek-Kozlina, B., Toxicity Assessment of heavy metal mixtures by *Lemna minor* L. *Sci Total Environ* 2007, 384(1-3), 229-238.
 - (35) Davidson, J.; Good, C.; Welsh, C.; Brazil, B.; Summerfelt, S., Heavy metal and waste metabolite accumulation

- and their potential effect on rainbow trout performance in a replicated water reuse system operated at low or high system flushing rates. *Aquac Eng* 2009, 41(2), 136-145.
- (36) Moens, L. M.; Smolders, R.; van der Ven, K.; van Remortel, P.; Del-Favero, J.; De Coen, W. M., Effluent impact assessment using microarray-based analysis in common carp; A systems toxicology approach. *Chemosphere* 2007, 67(11), 2293-2304.
- (37) Sabalinas, D.; Lazutka, J. R.; Sabaliuniene, I., Acute toxicity and genotoxicity of aquatic hydrophobic pollutants sampled with semipermeable membrane devices. *Environ Pollut* 2000, 109(2), 251-265.
- (38) Sakai, Y.; Shoji, R.; Kim, B. S.; Sakoda, A.; Suzuki, M., Cultured human-cell-based bioassay for environmental risk management. *Environ Monit Assess* 2001, 70(1-2), 57-70.
- (39) Oh, S. M.; Park, K.; Chung, K. H., Combination of in vitro bioassays encompassing different mechanisms to determine the endocrine-disrupting effects of river water. *Sci Total Environ* 2006, 354(2-3), 252-264.
- (40) Dewhurst, R. E.; Wheeler, J. R.; Chummun, K. S.; Mather, J. D.; Callaghan, A.; Crane, M., The comparison of rapid bioassays for the assessment of urban groundwater quality. *Chemosphere* 2002, 47(5), 547-554.

3

APPLICATION OF DNA MICROARRAY-BASED TRANSCRIPTOME ANALYSIS TO EVALUATION OF WHOLE WASTEWATER EFFLUENT IMPACTS ON HEPG2

OBJECTIVE

Due to its rapid, sensitive and comprehensive nature, the DNA microarray-based transcriptome analysis was applied to evaluate the impacts of whole wastewater effluents from the membrane bioreactors (MBRs) and the activated sludge process (AS), on the biological processes of human hepatoma HepG2 cells. The conventional bioassays (i.e., cytotoxicity tests and bioluminescence inhibition test) were conducted in parallel since they were well-established evaluation methods for the overall effluent toxicity. HepG2 was selected because it has been frequently used in vitro model for human detoxification of chemicals, is easy to handle, and provides a reproducible human system. However, since it is known that expression of specific genes were extremely low in the cancer derived HepG2, the results could not be simply extrapolated into the normal cells and in vivo (1). The present results altogether suggested the potential applicability of DNA microarray-based transcriptome analysis to impact evaluation of whole wastewater effluents.

MATERIALS and METHODS

Cell Culture. Human hepatoma HepG2 cells were provided by Cell Bank, RIKEN BioResource Center (Tsukuba, Japan). Cells were grown in Eagle's minimal essential medium (MEM) (Nissui, Tokyo, Japan), supplemented with 10% fetal bovine serum (FBS) and 60 mg/mL kanamycin. Cells were maintained at 37 °C in the 5% CO₂ humidified incubator.

Sampling site. Sampling was carried out from October 2011 to February, 2012 at the full-scale municipal wastewater treatment plant (Soseigawa Municipal Wastewater Treatment Plant, Sapporo, Japan), which daily received ca. 130,000 m³ of wastewater via combined sewage system. The plant uses an activated sludge system (AS), which consists of aeration tank and secondary sedimentation basin. Three pilot-scale MBRs were also installed at the plant: two submerged MBRs (S-MBRs) equipped with hollow-fiber polytetrafluoroethylene (PTFE) membrane with 0.3 µm nominal pore size, and one air-sparged side-stream MBR (AS-MBR) equipped with tubular polyvinylidene difluoride (PVDF) membrane with 0.03 µm nominal pore size (**Fig. 3-1**) (2). Two S-MBRs (S-MBR_A and S-MBR_B) were operated at different sludge concentration (mixed liquor suspended solids: MLSS) and solid retention times (SRTs). The operational conditions of selected processes are listed in **Table 3-1**. Raw wastewater was fed through a grit chamber to the MBRs.

Grab samples were taken around 10:00 am, from the effluents of the grid chamber (considered as raw wastewater), the secondary sedimentation basin of the AS, and the three MBRs. As a reference, two types of drinking waters were also collected: the tap water of our laboratory and the bottled water (commercial dechlorinated drinking water). In terms of general water quality, effluents from AS-MBR, S-MBRs and AS all satisfied the 40 national effluent standards (see "Supplemental information S1"). Characteristics of the raw wastewater of this plant can be found elsewhere (3).

Sample preparation and treatment. Immediately after sampling, 400 mL of collected samples were filtered with 0.22 µm polyethersulfone membrane using an aseptic vacuum filter system (Corning Japan K.K., Tokyo, Japan) for sterilization, and stored at 4 °C in sterilized muffled bottles until they were used for the assays (up to 48 h). Prior to exposure, 36 mL filtered water was combined with 4 mL of ×10 concentrated MEM, and supplemented with 1% fetal bovine serum (FBS) and 60 mg/mL kanamycin. Finally, pH was adjusted to 7.2-7.5 by adding 7.5% sodium hydrogen carbonate solution. Cells were seeded onto culture vessels in 10⁵ cell/mL and incubated overnight to grow 40-50% confluent. The normal culture medium was replaced by the prepared water samples and incubated for 48 h.

Table 3-1 Operational condition of the selected treatment processes. Two S-MBRs (S-MBRA and S-MBRB) were operated at different MLSS and SRT.

	AS-MBR	S-MBRA	S-MBRB	AS
HRT (h)	4	5	5	12
MLSS (g/L)	12	1	8	1.7
SRT (day)	40	10	50	7

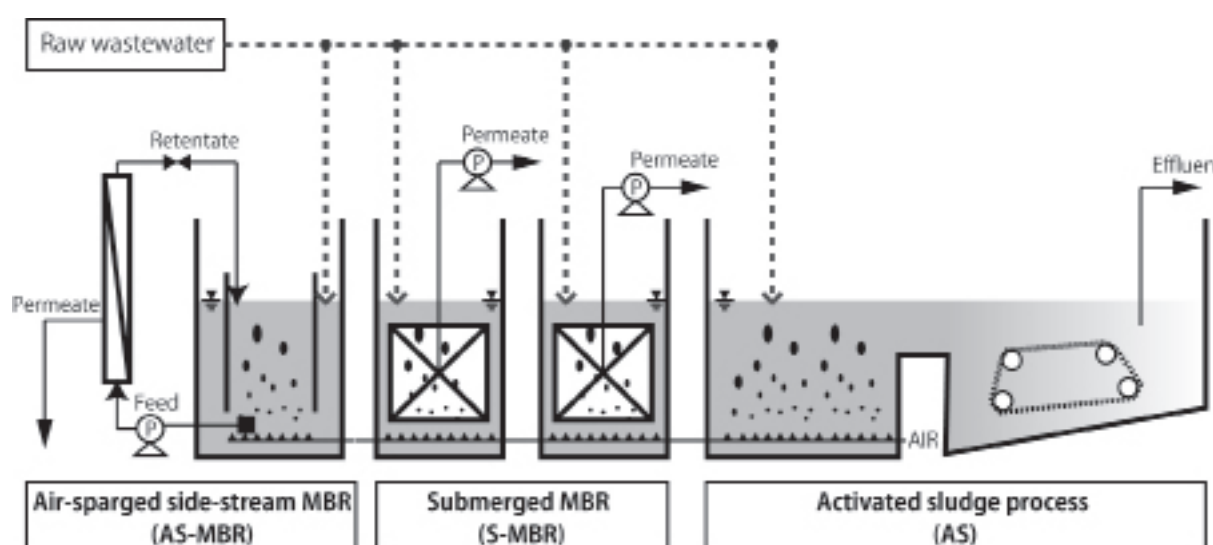


Fig. 3-1 Flow scheme of selected treatment processes. The raw wastewater and effluents were taken from October 2011 to February 2012 at a full-scale activated sludge process (AS) and three pilot-scale membrane bioreactors (MBRs) treating the same domestic wastewater

DNA microarray analysis. Cells were lysed on the 60 mm culture dishes, and total RNA was extracted using RNeasy Mini Kit (Qiagen, Hilden, Germany). For duplicate, two dishes were prepared for each sample and individually treated in parallel. Total RNAs were quantified using the Nanoview (GE Healthcare, US). The integrity of the RNA samples was also checked by microchip electrophoresis using the Cosmo Eye with the i-RNA Kit (Hitachi Chemical, Tokyo, Japan). RNA with a A260/A280 ratio 1.8 or higher was used for DNA microarray analysis using Human Genome Focus Array (Affymetrix, Santa Clara, CA), which represents 8,795 verified human sequences from the NCBI RefSeq database. Target preparation and hybridization were carried out as described in the manufacturer's instruction. After hybridization, the array was washed, stained with streptavidin-phycoerythrin using the Affymetrix GeneChip Fluidics Station 450 (Affymetrix), and scanned on a GeneChip Scanner 3000 7G (Affymetrix). The raw data files [cell intensity (CEL) files] were created from scanned images by AGCC (GeneChip Command Console Software, Affymetrix). The files are available on Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.gov/geo>) of the National Center for Biotechnology Information (NCBI) (accession number: GSE 45605).

Quantitative Real-Time Reverse-Transcriptase PCR (qPCR). Based on the microarray data, seven genes (i.e., CYP39A1, AKR1D1, STS, ADM, DUSP1, GCLC, and AQP3) were selected as marker genes from its differentially expressed genes associated with one or more of the biological processes such as “lipid metabolism” (i.e., CYP39A1, AKR1D1, STS, ADM), “response to endogenous stimulus” (i.e., STS, ADM, DUSP1, GCLC), and “response to inorganic substance” (i.e., DUSP1, GCLC, AQP3). IGFBP7 was used as the negative marker, which was differentially expressed only in AS exposure in the microarray analysis. The expressions of candidate marker genes were quantified by the quantitative real-time reverse-transcriptase polymerase chain reaction (qPCR) assay for the same RNA sample used for DNA microarray analysis, and those taken on different sampling days between October 2011 and August 2012 to confirm the reproducibility. Briefly, single-strand cDNA was synthesized from 25 ng/μL total RNA in reaction cocktail of PrimeScript RT reagent Kit (Takara, Shiga, Japan). The qPCR was carried out with ABI prism 7500 (Applied Biosystems, Foster City, CA) using SYBR Ex Taq II (Takara, Shiga, Japan), according to manufacturer’s instruction. The amplification reaction consisted of initial incubation at 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 30 s, annealing at specific temperatures for 30 s, and extension at 72°C for 60 s. The primer sequences and annealing temperatures are shown in Supplemental Information S4. Data were analyzed by the relative quantification method using the Ct values normalized with the endogenous reference, GAPDH, which was measured in all runs. The fold induction/suppression of each target gene was calculated by dividing the normalized Ct value in a sample with that of the control. All assays were tested for presence of unspecific amplicons and primer dimmers by melting curve analysis.

Statistics. For microarray analysis, probe-level data in CEL files were normalized by MAS5 using AGCC and transferred to the Subio Platform ver. 1.15 (Subio, Inc., Tokyo, Japan). Reproducibility of the overall gene expressions of sample pairs were confirmed based on physical distance by hierarchical clustering analysis and principal component analysis (PCA) (Supplemental Information S2). The features which were NOT flagged “Present” in any duplicates of the samples were rejected. We identified differentially expressed genes based on the two criteria: 1) a t-test p-value threshold of 0.05 and 2) a minimum fold change of 2 between the controls and exposed data sets. Gene ontology (GO) assignments and clustering into functional groups were performed using web-accessible programs provided by the Database for Annotation, Visualization and Integrated Discovery (DAVID) (<http://david.abcc.ncifcrf.gov/>).

Conventional bioassays. The three bioassays well-established for the evaluation of the overall effluent toxicity were performed in parallel with the DNA microarray analysis: bioluminescence inhibition test based on ISO 11348 and the two cytotoxicity tests using direct cell counting and MTT [3-(4,5-dimethylthial-2-yl)-2,5-diphenyltetrazolium bromide] assay. The procedures of the three bioassays are found in Supplemental Information S3.

RESULTS

Gene expression analysis using DNA microarray. The DNA microarray analysis identified 2 to 926 genes which were differentially expressed after exposure to the effluents and raw wastewater (**Table 3-2**). The numbers of the altered genes were far large in the raw wastewater (926 genes) compared to the effluents (<200 genes), indicating the reduction of cellular impacts after treatments. Among the effluents, however, the extent of alteration of the gene expression was relatively large for S-MBR_B (181 genes), moderate in S-MBR_A (39 genes) and AS (20 genes), and the lowest for AS-MBR (2 genes), which was even comparable to the tap water (TAP; 2 genes). For the bottled water (BOT), differentially expressed genes were not identified. More than half of the altered genes of S-MBR effluents were common with those of the raw wastewater.

Biological functional analysis was conducted by DAVID to connect altered genes and related biological processes, and identify the specific processes consisting of significant number of connected genes (**Table 3-3**). The smaller p value represents the higher significance of identification of the corresponding biological process. In AS-MBR effluent and the real drinking waters (i.e., TAP and BOT), any biological process was not identified at the statistical significance ($p < 0.05$).

For the rest of effluent samples and the raw wastewater, altered genes were categorized into 7 to 225 biological processes, which were further summarized into **major nine groups of closely related processes**: cell growth, cell division, lipid metabolism, lipid transport, response to endogenous stimulus, response to inorganic substance, acute inflammatory response, response to nutrients, and cell death. The biological processes identified for S-MBR_A exposure were common with those for S-MBR_B exposure, and the significance of alteration was mostly higher in the latter. The process groups such as “lipid metabolism” and “response to endogenous stimulus” were commonly identified in S-MBR effluents, AS effluent, and the raw wastewater exposures, while “response to inorganic substance” and “cell growth” were unique to S-MBR_B effluent and AS effluent, respectively. Although the significance of alteration of the gene expression was relatively high in “acute inflammatory response”, “cell death”, “response to nutrient” and “cell division”, these process groups were only identified in the raw wastewater.

Table 3-2 Number of differentially expressed genes in HepG2 cells after 48 hour exposure to effluents, the raw wastewater, the tap water (TAP), and the bottled water (BOT). Differentially expressed genes were selected based on the two criteria: 1) a t-test p-value threshold of 0.05 and 2) a minimum fold change of 2 between the controls and exposed data sets.

	AS-MBR	S-MBR _A	S-MBR _B	AS	TAP	BOT
Total	2	39	181	20	2	0
Induction	1	17	115	9	0	0
Suppression	1	21	66	11	2	0

Table 3-3 Gene Ontology (GO) categories of differentially expressed genes. Biological functional analysis was conducted by the web-accessible program, DAVID (<http://david.abcc.ncifcrf.gov/>) to assign GO terms to the altered genes and cluster the related biological processes.

Related Process Groups	Biological Process	p value (log10)				
		AS-MBR	S-MBR _A	S-MBR _B	AS	RAW
Cell growth	regulation of cell growth				-2.37	-1.50
	regulation of lipid metabolic process				-1.75	-1.16
Lipid metabolism	lipid catabolic process		-1.88	-1.95		
	steroid metabolic process		-2.23	-3.02		-6.02
	cholesterol metabolic process		-1.33	-1.05		-2.80
	bile acid catabolic process			-2.87		-1.49
	lipid transport			-1.73		-1.48
Lipid transport	carboxylic acid transport		-1.95	-1.12		
Response to endogenous stimulus	response to endogenous stimulus			-3.35	-1.39	-2.66
	response to hormone stimulus			-2.96		-2.77
	response to corticosteroid stimulus			-3.56		-1.68
	response to glucocorticoid stimulus			-3.07		-1.68
	response to estradiol stimulus					-2.07
Response to inorganic substance	response to inorganic substance			-1.40		
Acute inflammatory response	leukocyte mediated immunity					-2.93
Cell death	cell death					-2.45
	apoptosis					-2.49
Response to nutrient	response to nutrient					-2.45
	response to vitamin					-3.33
Cell division	mitosis					-10.01
	M phase					-9.37
	cell cycle					-8.24
	spindle organization					-3.47
	microtubule-based process					-3.31

Legend	-2.0 < log(p) ≤ -1.0
	-3.0 < log(p) ≤ -2.0
	-4.0 < log(p) ≤ -3.0
	log(p) ≤ -4.0

The altered genes in the selected process groups and their fold change (FC) (relative expression ratios to the control) for the effluents and the raw wastewater are listed in **Table 3-4**. Most of the genes related to “lipid metabolism” were shared between S-MBR effluents and raw wastewater, with the increase of FCs in the latter sample. In AS effluent, however, the altered genes such as AVPR1A, IGFBP7, and PPARA were unique to its exposure. The genes categorized in “response to endogenous stimulus” exhibited a similar trend as “lipid metabolism”, reflecting some overlaps of the genes and probably close relationship of the two functions in the cellular response to the effluents. In S-MBR_B effluent, the genes categorized in “response to inorganic substance” (i.e., DUSP1, FGB, SERPINE1, GCLC, and AQP3) were largely altered by 4- to 13-fold, while in AS effluent, the genes categorized in “cell growth”, (i.e., IGFBP7, CYR61, OSGIN1, and AVPR1A) were moderately altered by 2- to 4-fold.

Table 3-4 Differentially expressed genes in the selected process groups and their relative expression ratios (in base-2 logarithm).

(a) Lipid metabolism

Symbol	Gene name	Fold change (log2)				
		AS-MBR	S-MBR _A	S-MBR _B	AS	RAW
ADM	adenomedin		2.24			3.06
ADYT5N3	alk-1-like oxidoreductase family 1, member 5 (alk-1-like oxidoreductase)		2.43	2.27		2.07
AKR1C2	alk-1-like oxidoreductase family 1, member C2 (alk-1-like oxidoreductase 2; alk-1-like oxidoreductase, 3- α -hydroxysteroid dehydrogenase, type II)		1.68			2.18
APOL1	apolipoprotein L1			1.45		
AVPR1A	arginine vasopressin receptor 1A				2.11	
CYP2B41	cytochrome P450, family 2B, subfamily A, polypeptide 1		1.08	1.08		2.08
LEPR	leptin receptor			1.48		1.42
PPARA	peroxisome proliferator-activated receptor alpha				1.14	
AKR1D1	alk-1-like oxidoreductase family 1, member D1 (alk-1-like oxidoreductase 1-like oxidoreductase)	-1.76	-1.24			-3.32
APCS	apolipoprotein C-II			-1.87		-3.78
EHHA2H	enoyl-CoA hydratase/hydratase/CoA hydratase A dehydrogenase			-1.79		-3.70
ENPP2	ectonucleotide pyrophosphatase/phosphodiesterase 2	-1.85	-2.12			
IGFBP7	insulin-like growth factor binding protein 7				-1.15	-1.81
LDLR	low density lipoprotein receptor	-1.83				-1.52
NR1H4	nuclear receptor subfamily 1, group H, member 4			-1.81		-1.35
PLA2G1B	phospholipase A2, group II (pancreas)	-1.34	-1.76			-3.31
SERPINA6	serpin peptidase inhibitor, clade A (alpha-1 antitrypsin, antitrypsin), member 6			-1.70		-3.32
STS	sterol sulfatase (microsomal), isozyme B			-1.85		-2.36
SULT2A1	sulfotransferase family, cytochrome C, dehydroepiandrosterone (DHAP)-sulfonating, member 1			-1.29		-2.29
WDR20	WD domain containing oxidoreductase			-1.83		-1.53

(b) Response to endogenous stimulus

Symbol	Gene name	Fold change (log2)				
		AS-MBR	S-MBR _A	S-MBR _B	AS	RAW
ADCY7	adenylate cyclase 7		1.06	1.38		2.28
ADM	adenomedin			2.24		3.06
AVPR1A	arginine vasopressin receptor 1A				2.11	
CPN1	carboxypeptidase N, cytoplasmic 1			1.32		1.50
DUSP1	dual specificity phosphatase 1			2.08		2.63
ENC2	enkephalinase 2 (gamma, neuronal)			1.03		3.73
FHL2	four and a half LIM domain 2			1.04		2.11
FOSL1	FOS-like antigen 1			1.22		2.33
GCLC	glutamate-cysteine ligase, catalytic subunit			1.08		1.88
GN4G1	guanine nucleotide binding protein (G protein), alpha-activating activity polypeptide 1				2.36	
IGFBP1	insulin-like growth factor binding protein 1			1.86		2.18
IL6R	interleukin 6 receptor			1.02		1.70
LDLR	low density lipoprotein receptor			1.48		1.42
MMP3	matrix metalloproteinase 3 (stromelysin 1, stromelysin)				3.51	3.10
PLA2G1B	phospholipase A2, group II (pancreas)				2.20	
PRSS8	protease, serine, 8			1.86		2.03
AGAT	alanine glyoxylate aminotransferase			-1.37		-3.16
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)			-1.03		-1.31
IGFBP7	insulin-like growth factor binding protein 7				-1.15	-1.81
PPARA	peroxisome proliferator-activated receptor alpha	-1.34	-1.76			-3.31
SERPINA7	serpin peptidase inhibitor, clade A (alpha-1 antitrypsin, antitrypsin), member 7	-1.36	-1.77			-4.19
SLC142	solute carrier family 1 (glutamate high affinity glutamate transporter), member 2			-1.81		-2.36
STS	sterol sulfatase (microsomal), isozyme B			-1.85		-2.36

Table 3-4 Differentially expressed genes in the selected process groups and their relative expression ratios (in base-2 logarithm). (continued)

(c) Response to inorganic substances

Symbol	Gene name	Fold change (log2)				
		AS-MBR	S-MBR _A	S-MBR _B	AS	RAW
<i>AQP3</i>	aquaporin-3 [MTR group]			2.17		3.61
<i>AVPR1A</i>	arginine vasopressin receptor 1A				2.11	
<i>CDKN1A</i>	cyclin-dependent kinase inhibitor 1A (p21, Cip1)			1.32		1.50
<i>DUSP1</i>	dual specificity phosphatase 1			2.06		2.63
<i>FGSL1</i>	FGS-like antigen 1			1.22		2.33
<i>GCLC</i>	glutamate-cysteine ligase, catalytic subunit			1.98		1.86
<i>GNAO1</i>	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide 1				2.35	
<i>SERPINE1</i>	serpin peptidase inhibitor, clade C (hevin, plasminogen activator inhibitor type 1), member 1	1.72	3.68			5.80
<i>FGA</i>	fibronectin alpha chain	-1.81	-1.51			-5.75
<i>FBG</i>	fibronectin beta chain	-1.68	-2.43			-6.01
<i>FGS</i>	fibronectin gamma chain					-4.59
<i>MTX</i>	methotrexate			-1.50	-1.34	2.07

(d) Cell growth

Symbol	Gene name	Fold change (log2)				
		AS-MBR	S-MBR _A	S-MBR _B	AS	RAW
<i>AVPR1A</i>	arginine vasopressin receptor 1A				2.11	
<i>OSGIN1</i>	oxidative stress induced growth inhibitor 1	2.14			1.10	1.08
<i>IGFBP7</i>	insulin-like growth factor binding protein 7				-1.15	-1.81
<i>CYR61</i>	cysteine-rich angiogenic inducer 61				-1.31	

Quantification of candidate marker genes using qPCR. Eight genes were selected as representative of gene expression responses observed for the S-MBR_B effluent exposure, and their expression levels were quantified by qPCR assay (data shown in Supplemental Information S4). The FCs in the base-2 logarithm for all the selected genes varied from -3.3 to 3.6 in the microarray data, while those for qPCR were somewhere between -4.1 and 4.0. The direction of regulation (induction or suppression) was identical between the qPCR data and the microarray data. Although the more samples from different days are needed for meaningful statistical analysis, similar trend of overall gene expression patterns was observed for the all effluents taken on different days (**Fig 3-2**). The gene expression level of each gene varied day to day possibly due to the variation of water quality of effluents and the raw wastewater.

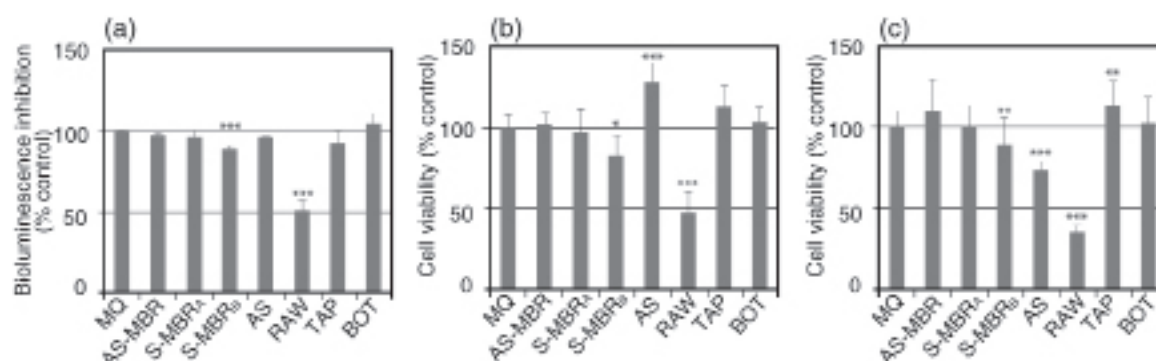


Fig. 3-3 Results of effluent toxicity evaluation by conventional bioassays: bioluminescence inhibition test (a) and the two cytotoxicity tests using direct cell counting (b) and MTT assay (c). Values were expressed as mean $\pm$ standard deviation of replicated experiments ($n > 3$). Statistical test was performed by using Tukey's multivariate analysis; *: <0.05; **: <0.01; ***: <0.001.

Bioluminescence inhibition. Bioluminescence intensity of *V. fischeri* after exposure to effluent samples, the raw wastewater and drinking water samples is shown in **Fig. 3-3a**. Clear bioluminescent inhibition was observed for raw wastewater (> 50% of the control). However, in AS-MBR, S-MBR_A, and AS effluents, the bioluminescence intensity was unchanged as compared to that of the control. Only S-MBR_B effluent showed a slight reduction (i.e., 10%). Similarly, significant bioluminescence inhibition was not observed for the drinking water samples.

Cytotoxicity. Cell viability was evaluated by direct cell counting with tripan blue dye exclusion method and the MTT assay (Fig. 3-3b and 3-3c). The direct cell counting exhibited a significant reduction of cell viability in S-MBR_B and raw wastewater by 13% and 55%, respectively, while AS-MBR and S-MBR_A effluents did not affect the cell viability. Cell viability increased by 26% after exposure to the AS effluent. The results of MTT assay were consistent to those of the direct cell counting except for AS, which on the contrary marked 23% reduction in the cell viability. The relative MTT activity divided by the cell numbers was smaller in AS effluent exposure (i.e., 0.58) even than in raw wastewater exposure (i.e., 0.74), suggesting its lower activity level of the HepG2 (4).

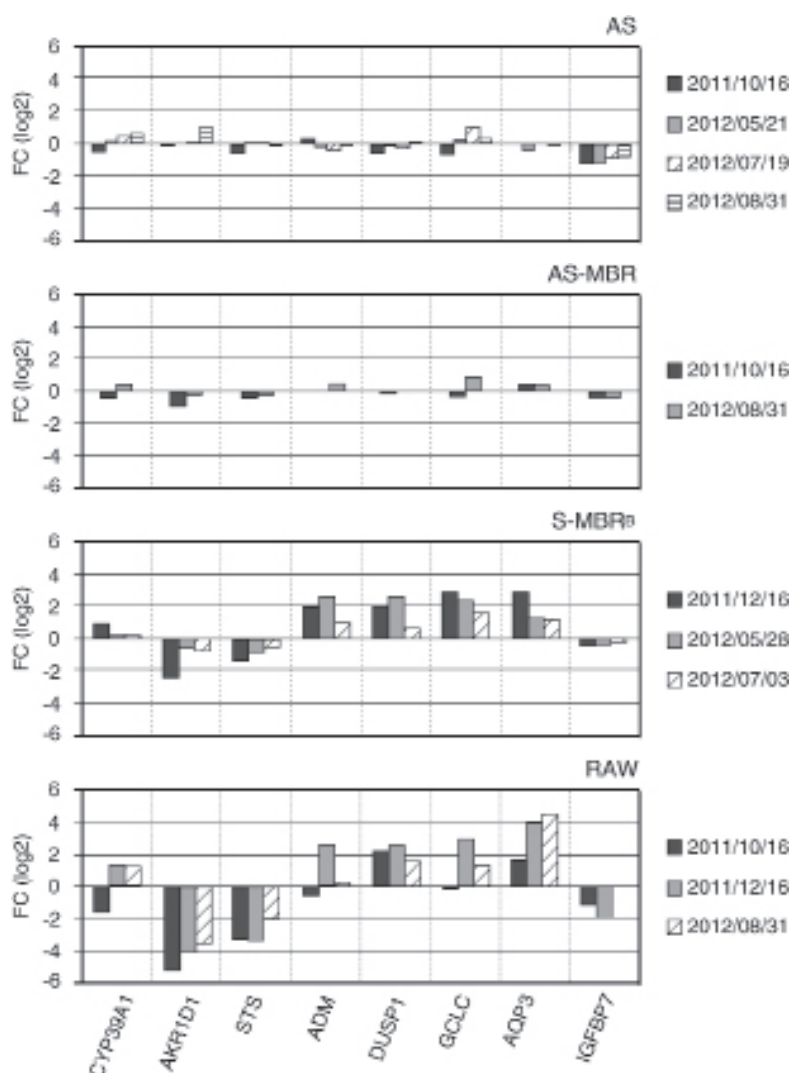


Fig. 3-2 Change of relative expression ratios (in base-2 logarithm) of eight selected genes in HepG2 cells exposed to effluents and the raw wastewater taken on different days. The expression level of eight genes was quantified by qPCR

DISCUSSION

Previous studies have demonstrated that global gene expression analysis using DNA microarray is a powerful tool to characterize the biological impacts of various environmental contaminants. These contaminants include benzene (5), dioxins and PCB (6), VOC (7), estrogens (8), pesticides (9), nanomaterials (10,11), arsenic (12,13) and heavy metals (14,15,16). However, applications of this technology to environmental samples or complicate mixtures of the contaminants are still limited. Up to now, only three studies have been reported the applications to wastewaters: industrial wastewater studied with common carp (17), wastewater from oil and gas production with zebrafish (18), and textile mill effluents with yeast (19). Previous studies mainly **targeted on the ecological impacts of industrial wastewaters, and the impacts of municipal wastewaters on humans** have not been investigated. In the present study, we applied for the first time this transcriptome analysis using the DNA microarray to human HepG2 cells exposed to treated and untreated municipal wastewaters for evaluation of whole effluents impacts on humans from the viewpoint of possible potable reuse.

Table 3-5 summarizes the results of the transcriptome analysis and the three conventional bioassays applied to the MBR effluents, the AS effluent, the raw wastewater, and the real drinking waters (i.e., TAP and BOT). Despite that all the treated effluents satisfied the 40 national effluent standards; transcriptome analysis of HepG2 exposed to the effluents demonstrated a variation in the effluent quality in terms of their impacts on cellular processes (**Table 5-3**). The degree of impacts on the gene expression **was roughly evaluated based on the number and the degree of differentially expressed genes and the affected biological processes as S-MBR_B>S-MBR_A>AS>AS-MBR**. This result suggested that the effluent from S-MBR_B had the largest impacts on the HepG2 cells and the AS-MBR had the least. The three conventional bioassays (i.e., direct cell counting, MTT assay, and the bioluminescence inhibition test) supported the result of transcriptome analysis. In both direct cell counting and MTT assay, only S-MBR_B effluent showed a reduction of cell viability. These results suggested that the overall or a part of observed gene expression responses could be related to adverse effects on the cell proliferation in the sub-lethal region.

Table 5 Comparison of results for transcriptome analysis, conventional bioassays, and chemical analysis of effluent standards.

	AS-MBR	S-MBR _A	S-MBR _B	AS	RAW	TAP	BOT
① TSB assay							
No. of differentially expressed genes	2	39	181	20	926	2	0
No. of affected biological processes	0	12	57	7	225	0	0
② Conventional bioassays							
Bioluminescence inhibition test	—	—	+	—	++	—	—
Direct counting	—	—	+	—	++	—	—
MTT assay	—	—	+	+	++	—	—
③ Chemical analysis							
Effluent standards	—	—	—	—	N/A	N/A	N/A

— : satisfactory or <10% reduction, + : 10-30% reduction, ++ : >50% reduction, N/A: not applied

Although we currently cannot exclude the genetic response derived from the innocuous matters, if any, the changes of biological processes which have been suggested as the response to the toxic chemicals or environmental stresses were considered as a potential indication for the toxic effects. Among the identified biological processes, “lipid metabolism” was the only process commonly affected by the exposure to the effluents and the raw wastewater. The alteration of the genes in this biological process was also reported when HepG2 cells were exposed to the organic toxicants such as aflatoxin (20), polycyclic aromatic hydrocarbons (PAHs) (21,22), cationic amphiphilic drugs (23), and also the common carp exposed to the oil contaminated water (17). Based on these results, it was inferred that the alteration of “lipid metabolism” might not be a chemical-specific response but a general response to a wide range of chemicals in the tested cells. The drastic alteration of the lipid metabolism-related genes in the raw wastewater supported this hypothesis since the raw wastewater was likely to contain the largest variety of chemicals as indicated in its highest organic content (total organic carbon (TOC): 34 mg/L). The alteration of cellular function related to lipid metabolism, therefore, could be proposed as a potential indication of effluent impacts in general, and the genes categorized in this process (i.e., CYP39A1, AKR1D1, STS, ADM) may serve as its marker genes.

Besides, the functional analysis of the altered genes indicated that the S-MBR_B effluent and the AS effluent had their own unique impacts on the cells. In the S-MBR_B exposure, the group of genes categorized in “response to inorganic substance” such as DUSP1, FGB, SERPINE1, GCLC, and AQP3 were significantly altered by 4-13 folds (**Table 3-4**). Previous studies, for example, have reported AQP3 was related to the transport of arsenite into mammalian cells (24,25), while the glutathione level affected by GCLC expression has been demonstrated to play a pivotal role in excretion of heavy metals (26). Induction of the genes associated with ion transport (particularly iron) was reported for yeast and zebrafish exposed to industrial wastewaters (18,19). Alteration of the genes associated with “response to inorganic substance” along with those associated with “lipid metabolism” in the S-MBR_B exposure suggested the existence of residual organic and inorganic contaminants in the effluent, while AS, AS-MBR, and S-MBR_A exhibited gene expression response only related to the organics.

The wastewater effluents contain vast categories of chemicals including unknown compounds, and their dose-response relationship in such a complex matrix has not yet been fully understood. In this sense, it is difficult, and at the same time, less fruitful to identify individual constituents which might be responsible for the alteration of gene expression. To improve the public acceptance for reuse of the reclaimed wastewater, the potential toxicity of whole effluent must be screened, and the toxicity reduction measures need to be selected. For this purpose, the genes discussed above could be used as marker genes. Thus, it is of greater importance that indicators of harmful impacts are to be distinguished from innocuous ones, and this is where more intensive research is necessary.

To be noted, transcriptome analysis together with the conventional bioassays indicated the smaller cellular impacts of S-MBR_A with shorter SRT (or lower MLSS) than S-MBR_B with longer SRT (or higher MLSS) even though all the tested MBRs were treating the same domestic wastewater. Previous researches have reported that the accumulated organic matters in the MBRs varied with SRTs such as the increase in hydrophobic humic or humin-like substances with longer SRT (27,28). Although a limited number of reports are currently available for the toxicity of effluent organic matters (EfOM) (29), we hypothesize that a certain type of EfOM produced only in the longer SRT (S-MBR_B) posed adverse effects on the tested cells, and currently the physicochemical properties of the biologically active fractions are being investigated. Compared between MBRs with similar SRTs, the AS-MBR with 0.03 μm membrane pore size exhibited much smaller impact than S-MBR_B with 0.3 μm membrane pore size, whose gene expression pattern was very similar to that of the raw wastewater (**Table 3-4**). These results suggested that the tighter membrane was effective to reject the responsible fraction for the biological impacts, verification of which is also the focus of our on-going research.

SUMMARY

In conclusion, the DNA microarray-based transcriptome analysis with human HepG2 cells was suggested as a powerful tool to rapidly and comprehensively evaluate impacts of whole wastewater effluents. The potential genetic markers were also

proposed to quantitatively evaluate the treatability of the wastewater effluents, which can be used to search for the physicochemical properties of the biologically active fraction of effluents. However, since the harmful impacts and innocuous impacts could not be distinguished at present, continued efforts are indispensable to identify some key biological processes and establish their relationship with some important endpoints.

REFERENCES

- (1) Wilkening, S.; Stahl, F.; Bader, A., Comparison of primary human hepatocytes and hepatoma cell line HepG2 with regard to their biotransformation properties. *Drug Metab Dispos* 2003, 31(8), 1035-1041
- (2) Hoque, A.; Kimura, K.; Miyoshi, T.; Yamato, N.; Watanabe, Y., Characteristics of foulants in air-sparged side-stream tubular. membranes used in a municipal wastewater membrane bioreactor. *Sep Purif Technol* 2012, 93, 83-91.
- (3) Kimura, K.; Yamato, N.; Yamamura, H.; Watanabe, Y., Membrane fouling in pilot-scale membrane Bioreactors (MBRs) treating municipal wastewater. *Environ Sci Technol* 2005, 39 (16), 6293-6299.
- (4) Ezendam, J.; Staedtler, F.; Pennings, J.; Vandebriel, R. J.; Pieters, R.; Harleman, J. H.; Vos, J. G., Toxicogenomics of subchronic hexachlorobenzene exposure in Brown Norway rats. *Environ Health Perspect* 2004, 112 (7), 782-791.
- (5) Gerlier, D.; Thomasset, N., Use of MTT colorimetric assay to measure cell activation. *J Immunol Methods* 1986, 94(1-2), 57-63.
- (6) Vezina, C. M.; Walker, N. J.; Olson, J. R., Subchronic exposure to TCDD, PeCDF, PCB126, and PCB153: Effect on hepatic gene expression. *Environ Health Perspect* 2004, 112 (16), 1636-1644.
- (7) Yim, W. C.; Min, K.; Jung, D.; Lee, B. M.; Kwon, Y., Cross experimental analysis of microarray gene expression data from volatile organic compounds treated targets. *Mol Cell Toxicol* 2011, 7 (3), 233-241.
- (8) Olsvik, P. A., K. K. Lie, et al. (2009). "Transcriptional effects of nonylphenol, bisphenol A and PBDE-47 in liver of juvenile Atlantic cod (*Gadus morhua*)."*Chemosphere* 2009, 75(3), 360-367.
- (9) Kitagawa, E.; Takahashi, J.; Momose, Y.; Iwahashi, H., Effects of the pesticide thiuram: Genome-wide screening of indicator genes by yeast DNA microarray. *Environ Sci Technol* 2002, 36 (18), 3908-3915.
- (10) Kawata, K.; Osawa, M.; Okabe, S., In Vitro Toxicity of Silver Nanoparticles at Noncytotoxic Doses to HepG2 Human Hepatoma Cells. *Environ Sci Technol* 2009, 43 (15), 6046-6051.
- (11) Kim, J. S.; Song, K. S.; Lee, J. K.; Choi, Y. C.; Bang, I. S.; Kang, C. S.; Yu, I. J., Toxicogenomic comparison of multi-wall carbon nanotubes (MWCNTs) and asbestos. *Arch Toxicol* 2012, 86 (4), 553-562.
- (12) Hamadeh, H. K.; Trouba, K. J.; Afshari, C. A.; Germolec, D., Coordination of altered DNA repair and damage pathways in arsenite-exposed keratinocytes. *Toxicol Sci* 2002, 69 (2), 306-316.
- (13) Su, P. S.; Hu, Y. J.; Ho, I. C.; Cheng, Y. M.; Lee, T. C., Distinct gene expression profiles in immortalized human urothelial cells exposed to inorganic arsenite and its methylated trivalent metabolites. *Environ Health Perspect* 2006, 114 (3), 394-403.
- (14) Andrew, A. S.; Warren, A. J.; Barchowsky, A.; Temple, K. A.; Klei, L.; Soucy, N. V.; O'Hara, K. A.; Hamilton, J. W., Genomic and proteomic profiling of responses to toxic metals in human lung cells. *Environ Health Perspect* 2003, 111 (6), 825-838.
- (15) **Kawata, K.; Yokoo, H.; Shimazaki, R.; Okabe, S., Classification of heavy-metal toxicity by human DNA microarray analysis. *Environ Sci & Technol* 2007, 41 (10), 3769-3774.**
- (16) Kawata, K.; Shimazaki, R.; Okabe, S., Comparison of gene expression profiles in HepG2 cells exposed to arsenic, cadmium, nickel and three model carcinogens for investigating the mechanisms of metal carcinogenesis. *Environ Mol Mutagen* 2009, 50, 46-59
- (17) Moens, L. M.; Smolders, R.; van der Ven, K.; van Remortel, P.; Del-Favero, J.; De Coen, W. M., Effluent impact assessment using microarray-based analysis in common carp; A systems toxicology approach. *Chemosphere* 2007, 67(11), 2293-2304.
- (18) Olsvik, P. A.; Lie, K. K.; Stavrum, A.; Mier, S., Gene-expression profiling in gill and liver of zebrafish exposed to produced water. *Int J Environ Anal Chem* 2007, 87(3), 195-210.
- (19) Kim, H.; Rakwal, R.; Shibato, J.; Iwahashi, H.; Choi, J. S.; Kim, D. H., Effect of textile wastewaters on

- Saccharomyces cerevisiae* using DNA microarray as a tool for genome-wide transcriptomics analysis. *Water Res* 2006, 40(9), 1773-1782.
- (20) Ratajewski, M.; Walczak-Drzewiecka, A.; Salkowska, A.; Dastych, J., Aflatoxins upregulate CYP3A4 mRNA expression in a process that involves the PXR transcription factor. *Toxicol Lett* 2011, 205 (2), 146-153.
- (21) Castorena-Torres, F.; de Leon, M. B.; Cisneros, B.; Zapata-Perez, O.; Salinas, J. E.; Albores, A., Changes in gene expression induced by polycyclic aromatic hydrocarbons in the human cell lines HepG2 and A549. *Toxicol in Vitro* 2008, 22 (2), 411-421.
- (22) Staal, Y. C. M.; van Herwijnen, M. H. M.; van Schooten, F. J.; van Delft, J. H. M., Modulation of gene expression and DNA adduct formation in HepG2 cells by polycyclic aromatic hydrocarbons with different carcinogenic potencies. *Carcinogenesis* 2006, 27 (3), 646-655.
- (23) Sawada, H.; Takami, K.; Asahi, S., A toxicogenomic approach to drug-induced phospholipidosis: Analysis of its induction mechanism and establishment of a novel in vitro screening system. *Toxicol Sci* 2005, 83 (2), 282-292.
- (24) Wang, J. L., D. P. Yin, et al. (2007). "Dual specificity phosphatase 1/CL100 is a direct transcriptional target of E217-1 in the apoptotic response to oxidative stress." *Cancer Res* 67(14): 6737-6744.
- (25) Naranmandura, H., Y. Ogra, et al. "Evidence for toxicity differences between inorganic arsenite and thioarsenicals in human bladder cancer cells." *Toxicol and Appl Pharmacol* 2009, 238(2): 133-140.
- (26) Aiba, I.; Hossain, A.; Kuo, M. T., Elevated GSH level increases cadmium resistance through down-regulation of Sp1-dependent expression of the cadmium transporter ZIP8. *Mol Pharmacol* 2008, 74 (3), 823-833.
- (27) Liang, S.; Liu, C.; Song, L. F., Soluble microbial products in membrane bioreactor operation: Behaviors, characteristics, and fouling potential. *Water Res* 2007, 41 (1), 95-101.
- (28) Kimura, K.; Naruse, T.; Watanabe, Y., Changes in characteristics of soluble microbial products in membrane bioreactors associated with different solid retention times: Relation to membrane fouling. *Water Res* 2009, 43 (4), 1033-1039.
- (29) Barker, D. J.; Stuckey, D. C., A review of soluble microbial products (SMP) in wastewater treatment systems. *Water Res* 1999, 33 (14), 3063-3082

4

EVALUATION OF DOSE-RESPONSE RELATIONSHIP OF SELECTED GENES AND DEVELOPMENT OF EFFLUENT-RESPONSIVE MARKER GENES

OBJECTIVE

To develop marker genes which respond to wastewater effluent impacts, the DNA microarray was first applied to HepG2 cells exposed to concentrated MBR and AS effluents at the same organic concentration (i.e., 30 mg-DOC/L), and selected genes were further quantified by quantitative RT-PCR assay for various concentrations. The concentration of effluent samples was achieved by filtration with reverse osmosis (RO) membrane. Moreover, the uniqueness of the gene expression profiles of the effluents were investigated in comparison to model toxicants (i.e., heavy metals, oxidative stress inducers, and carcinogens).

MATERIALS and METHODS

Cell Culture. Human hepatoma HepG2 cells were provided by Cell Bank, RIKEN BioResource Center (Tsukuba, Japan). HepG2 was selected because it has been frequently used in vitro model for human detoxification of chemicals, is easy to handle, and provides a reproducible human system. However, since it is known that expression of specific genes were extremely low in the cancer derived HepG2, the results could not be simply extrapolated into the normal cells and in vivo (1). Cells were grown in Eagle's minimal essential medium (MEM) (Nissui, Tokyo, Japan), supplemented with 10% fetal bovine serum (FBS) and 60 mg/mL kanamycin. Cells were maintained at 37 °C in the 5% CO₂ humidified incubator.

Sampling site. Sampling was carried out in December 2012 and February 2013 at the pilot-scale MBR plant treating the real municipal wastewater collected via combined sewage system in Sapporo, Japan. Two pilot-scale MBRs were installed at the plant: the submerged MBR (S-MBR) equipped with hollow-fiber polytetrafluoroethylene (PTFE) membrane with 0.3 µm nominal pore size, and the air-sparged side-stream MBR (AS-MBR) equipped with tubular polyvinylidene fluoride (PVDF) membrane with 0.03 µm nominal pore size (2). The operational conditions and general effluent quality of selected processes are found in elsewhere (3). Characteristics of the raw wastewater of this plant can be found elsewhere (4). Grab samples were taken from the two MBRs around 10:00 am.

RO concentration and pretreatment. Immediately after sampling, the MBR effluents were pre-filtered with 0.22 µm PTFE membrane (Advantec Toyo Kaisha, Ltd., Tokyo, Japan) to remove suspended solids. Effluent concentration by reverse osmosis (RO) was conducted by the cross-flow filtration system for flat-sheet membranes (SEPA CF II Unit; SANKO Ltd., Yokohama, Japan), equipped with polyamide RO membrane (effective surface area: 140 cm²; GE Water & Process Technologies, PA, US) under the constant pressure (2 MPa). The cross-flow speed and permeate flux were 0.1 m/sec and 42 L/m²/h, respectively. During the operation over two nights, the retentate was kept in cold water bath (< 8 °C), and the salt rejection rate (> 98%) was monitored by the electronic conductivity meter.

The concentrate was again filtered for sterilization and dissolved organic carbon (DOC) was measured by the total organic carbon (TOC) analyzer (Shimadzu, Shiga, Japan). After adjustment of DOC to six levels from original DOC up to 50 mg/L using sterilized Milli-Q water, 46 mL of each dilution was combined with 4 mL of ×10 concentrated MEM, and supplemented with 1% fetal bovine serum (FBS) and 60 mg/mL kanamycin. Finally, pH was adjusted to 7.2-7.5 by adding 7.5% sodium hydrogen carbonate solution. Cells were seeded onto culture vessels in 10⁵ cell/mL and incubated overnight to grow 40-50% confluent. The normal culture medium was replaced by the prepared samples and incubated for 48 h.

Cytotoxicity test. Cytotoxic effects of effluents on HepG2 cells were studied after 48 hour exposure based on mitochondrial dehydrogenase activity. The mitochondrial dehydrogenase activity was determined by using MTT assay, which measured the amount of formazane resulted from reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT). MTT (Sigma-Aldrich, Japan) dissolved in PBS was added onto the treated cells seeded in 96-well plates and incubated for 4 hours. Supernatant was removed after centrifugation for 5 min at 3000 rpm, and the amount of formazane pigment in DMSO (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) elutes was measured at 540 nm by plate reader (multilabel

counter ARVO MX, PerkinElmer, MA, US).

DNA microarray. The DNA microarray-based transcriptome analysis has been used as a comprehensive, rapid and sensitive tool to detect the genome-wide gene expressions, whose patterns represent the primary interactions between the constituents of effluents and biota, and widely applied for environmental pollutants (5,6,7,8). Cells exposed to the concentrated S-MBR effluent were lysed on the 60 mm culture dishes, and total RNA was extracted using RNeasy Mini Kit (Qiagen, Hilden, Germany). For duplicate, two dishes were prepared for each sample and individually treated in parallel. Total RNAs were quantified using the Nanoview (GE Healthcare, US). The integrity of the RNA samples was also checked by microchip electrophoresis using the Cosmo Eye with the i-RNA Kit (Hitachi Chemical, Tokyo, Japan). RNA with a A260/A280 ratio 1.8 or higher was used for DNA microarray analysis using Human Genome Focus Array (Affymetrix, Santa Clara, CA), which represents 8,795 verified human sequences from the NCBI RefSeq database. Target preparation and hybridization were carried out as described in the manufacturer's instruction. After hybridization, the array was washed, stained with streptavidin-phycoerythrin using the Affymetrix GeneChip Fluidics Station 450 (Affymetrix), and scanned on a GeneChip Scanner 3000 7G (Affymetrix). The raw data files [cell intensity (CEL) files] were created from scanned images by AGCC (GeneChip Command Console Software, Affymetrix). The files will be available on Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.gov/geo>) of the National Center for Biotechnology Information (NCBI). Gene expression profiles obtained in our previous studies were used (ref, ref).

Statistics. For microarray analysis, probe-level data in CEL files were normalized by MAS5 using AGCC and transferred to the Subio Platform ver. 1.15 (Subio, Inc., Tokyo, Japan). Reproducibility of the overall gene expressions of sample pairs were confirmed based on physical distance by hierarchical clustering analysis and principal component analysis (PCA) (Supplemental Information B). The features which were NOT flagged "Present" in any duplicates of the samples were rejected. We identified differentially expressed genes based on the two criteria: 1) a t-test p-value threshold of 0.05 and 2) a minimum fold change of 2 between the controls and exposed data sets. Gene ontology (GO) assignments and clustering into functional groups were performed using web-accessible programs provided by the Database for Annotation, Visualization and Integrated Discovery (DAVID) (<http://david.abcc.ncifcrf.gov/>). The hierarchical clustering (using Pearson distance metric) analysis and principal component analysis (PCA) were performed on the genes flagged "Present" in any one or more samples.

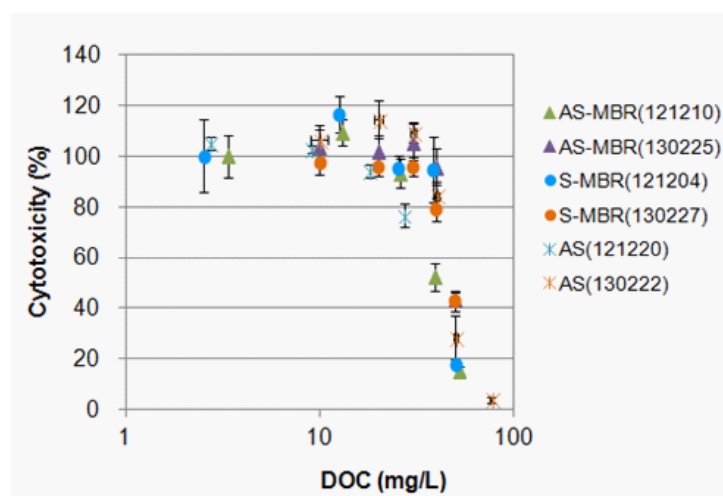
RESULTS

Performance of RO. MBR and AS effluents were concentrated by RO at the cross-flow constant-pressure mode. Enrichment factor and recovery ratio of organic matters by RO are shown in **Table 4-1**. Organic contents of AS-MBR and S-MBR effluents were concentrated more than 10 and 12 times with more than 87% and 80% of recovery in terms of DOC, respectively, while the factor of AS effluents was about 10 times or higher with the recovery more than 80%. The recovery observed in this study was comparable to those reported in the previous researches (26). These results demonstrated that RO successfully enriched the much larger percentage of organic content of the MBR effluents (> 80%), compared to the solid phase extraction (SPE) with C18 or OASIS HLB sorbents (usually below 50%) at the neutral pH. Even though the RO concentration takes much longer time (2-3 days) than popular SPE methods (several hours), it could be an effective enrichment method when whole wastewater effluents with unknown content are to be evaluated, and thereby, surrogate standards for the unknown residues is difficult to determine.

Table 4-1 Concentration ratio and recovery of organic matters by RO

Effluent type	Sampling date	DOC recovery (%)	Enrichment factor (-)
AS-MBR	December 06, 2012	85.8	15.3
	February 25, 2013	83.5	10.0
S-MBR	December 13, 2012	87.6	13.5
	February 27, 2013	88.5	12.7
AS	December 20, 2012	80.4	9.8
	February 22, 2013	84.9	10.3

Cytotoxicity. Cell viability of HepG2 after 48 h exposure to MBR and AS effluents at various concentrations (i.e., from DOC level in original effluents to 50 mg/L) is shown in **Fig. 4-1**. In both MBR effluents and AS effluents, drastic reduction of cell viability was observed in the concentration range at 30 mg-DOC/L or higher, while in lower DOC region, cell viabilities did not change significantly. Cell viability of the sodium chloride solution which gave electronic conductivity equivalent to the highest concentrated MBR effluents (ca. 500 mS/m) were more than 80% (data not shown). This result suggested that major cause of cytotoxicity observed at 30 mg/L of DOC or higher was not the elevated osmotic pressures after RO concentration but adverse impacts related to the effluent constituents themselves. Cell viability of different effluents were similar at the same DOC levels, which might reflect the comprehensive nature of the cytotoxicity test, at the same time, its limitation in telling the difference of the effluent impacts.

**Fig 4-1** Cytotoxicity of concentrated effluents

Gene Expression Profile at sub-lethal concentration. Based on the result of the cytotoxicity test, 30 mg-DOC/L was selected for the DNA microarray analysis since it was considered as the maximum concentration, at which more than 80% cell viability was still determined (ref). **Table 4-2** summarizes the number of differentially expressed genes in HepG2 exposed to concentrated MBR and AS effluents, along with the breakdown of induced and suppressed genes, and the number of biological processes identified by functional analysis using DAVID. Exposure of effluents concentrated up to sub-lethal level (i.e., 30 mg/L) altered 103, 75, and 157 genes in AS-MBR, S-MBR, and AS effluents, respectively, which were further categorized into 28, 38, and 75 biological processes. Major biological processes previously identified for the non-concentrated effluents (e.g., lipid metabolism, response to endogenous stimulus, and response to inorganic substances) were

also found in the concentrated MBR and AS effluents in common. To be noted, it was revealed only in the present study with the concentrated effluent that S-MBR effluent and AS effluent significantly induced genes related to anti-oxidant reaction or xenobiotics metabolism, inferring the advantage of using sub-lethal concentration in the DNA microarray analysis. Moreover, these results indicated that the degree of the overall gene expression response not necessarily corresponded to the degree of cytotoxicity since the number of DEGs of the concentrated S-MBR effluents (i.e., 30 mg-DOC/L) were lower than those of the non-concentrated effluents (i.e., <5 mg-DOC/L).

Table 4-2 Number of differentially expressed genes and identified biological processes

Effluent type	Induction	Suppression	Total	Identified biological process (<0.05)
AS-MBR	61	42	103	28
S-MBR	36	39	75	38
AS	70	87	157	75

Fig. 4-2 shows the overlap of the DEGs among the three effluents. Four out of 17 genes commonly altered in all the three effluents, also ranked in the top ten genes with large FCs among the DEGs of each effluent. This result together with those of the GO analysis suggested that the impacts on HepG2 were similar among the selected MBR and AS effluents, and commonly altered genes could reflect the general effluent impacts. The induced genes are related to immune response (i.e., SAA4 and CD3D), G-protein coupled receptor signaling pathway (i.e., GPR109B), detoxification (i.e., GCLM and GPX2), anti-oxidant response (i.e., AKR1B10 and AKR1C2), and apoptosis and cell death (i.e., PLA2G1B, PLP1, PDZK1, FGA, and DAPK1), implying the presence of toxic or xenobiotic wastewater residues in the MBR and AS effluents, and induction of genes responding to them. CYP1A1, which was significantly induced only in S-MBR and AS effluents, is known to play a pivotal role in detoxification.

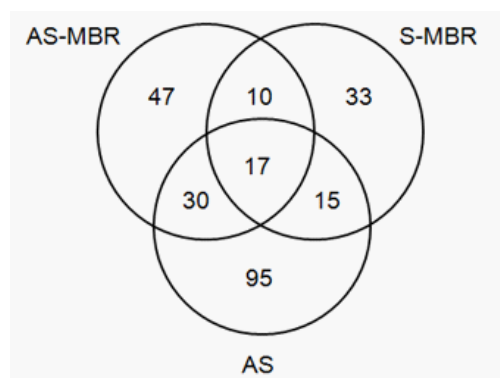


Fig 4-2 Cytotoxicity of concentrated effluents

Quantification of selected marker genes. The detection frequency of the genes described above, and their accuracy in the DNA microarray analysis were taken into consideration (**Table 4-3**), and nine genes were selected as candidate marker genes of wastewater impact. To examine their capability as biomarker, the gene expression levels were quantified by qPCR over six concentrations from original DOC in the effluent up to 50 mg/L. FCs (relative expression ratios to the control) of candidate marker genes are shown in the **Fig. 4-3**. CYP1A1, AKR1B10, GPX2, GCLM, and PDZK exhibited a clear concentration-dependency in their expression level changes, while it was only partly observed for AKR1C2 and GPR109B, and the correlation was poor for CD3D and PLA201B. Since the extent of the fluctuation was relatively small for PDZK, four genes (i.e., CYP1A1, AKR1B10, GPX2, and GCLM) were determined as marker genes.

Table 4-3 Commonly altered genes among MBR and AS effluents

[Induction]

	Gene Symbol	Fold change (FC)			Absolute call	Detection frequency	Selection
		AS-MBR	S-MBR	AS			
1	SPP1	10.29	4.91	4.44	-		
2	LOC55908	4.48	4.38	2.39	-		
3	SAA4	2.22	4.31	4.04	-		
4	CD3D	2.56	4.31	5.97	x	1	x
5	AKR1B10	4.82	3.70	3.75	x	6	x
6	GPR109B	4.02	3.46	2.35	x	1	x
7	GCLM	3.78	3.32	3.86	x	2	x
8	GPX2	3.17	3.28	3.25	x	5	x
9	AKR1C2	2.87	2.69	2.07	x	1	x
10	CSTA	2.47	2.45	2.44	-		

[Suppression]

	Gene Symbol	Fold change (FC)			Absolute call	Detection frequency	Selection
		AS-MBR	S-MBR	AS			
1	PLA2G1B	0.20	0.30	0.21	x	4	x
2	PLP1	0.21	0.30	0.15	-		
3	PDZK1	0.33	0.32	0.25	x	3	x
4	FGA	0.20	0.34	0.46	x	2	
5	GNMT	0.47	0.37	0.29	-		
6	HPR	0.35	0.38	0.35	x	1	
7	DAPK1	0.46	0.43	0.46	-		

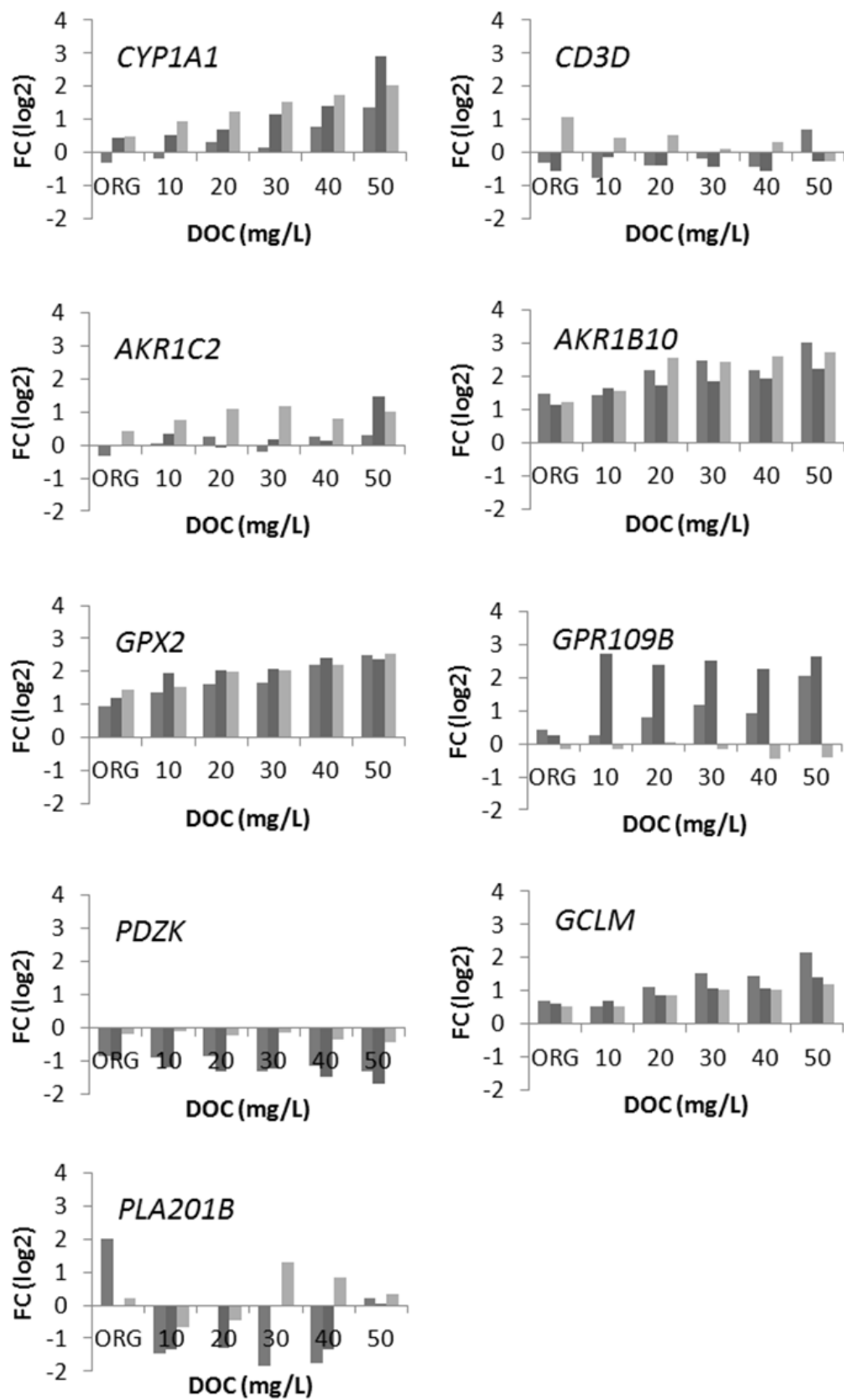


Fig. 4-3 Expression levels of candidate marker genes in HepG2 exposed to six different concentrations

Comparison of gene expression profiles between effluents and model chemicals. The principal component analysis (PCA) and hierarchical clustering analysis using the Pearson distance metric were performed on the data sets obtained for the concentrated S-MBR effluent in this study and for heavy metals (cadmium, manganese, nickel), metalloid (arsenic), tumor promoter (12-O-Tetradecanoylphorbol-13-Acetate: TPA), tumor initiator (dimethylnitrosamine: DMN), and oxidative stress inducing agent (2,3-dimethyloxy-1,4-naphto- quinine: DMNQ) in our previous studies (ref, ref), which were all tested using HepG2 cells in the same exposure condition (i.e., sub-lethal concentration for 48 hour) except for the tumor promoter, TPA (non-lethal exposure level). The PCA result is shown in **Fig. 4-4**. The contribution of 1st and 2nd component was 25.0% and 17.9%, respectively, which covered 43% of gene expression trends of over 8,500 genes on HG Focus microarray (Affimetrix). Gene expression profiles of the effluent and model chemicals were classified into three groups: 1) TPA, DMN and As, 2) heavy metals and DMNQ, and 3) S-MBR effluent. Indeed, the location of S-MBR effluent was distinct from the selected chemicals, suggesting the uniqueness of gene expression response in HepG2 cell toward the wastewater effluent.

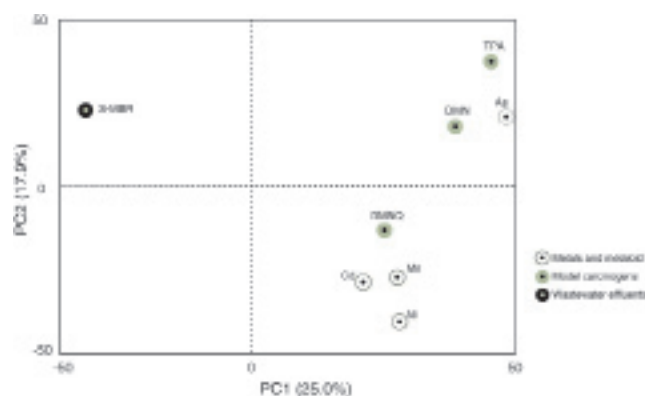


Fig. 4-4 Principal component analysis (PCA) of gene expression profiles of S-MBR effluent and model chemicals

The PCA result was further supported by the hierarchical clustering analysis (**Fig. 4-5**). The tree dendrogram showed that S-MBR formed independent cluster isolated from all the other tested chemicals. The differentially expressed genes found in the band showing the contrast gene expression trends between S-MBR and the other selected chemicals, included AKR1C2 and AKR1B10 identified in “steroid metabolic processes”, and PLP1, HAMP, and NR3C2 in “ion homeostasis”, and FGA related to “response to inorganic substances”. These results indicated that some genes categorized in “steroid metabolic process” and “response to inorganic substances” were potentially useful to characterize the effluents’ gene expression profiles which were significantly different from the metals, carcinogens, and oxidative stress inducer. In other words, characteristic features of those model toxicants were not found in the effluent sample.

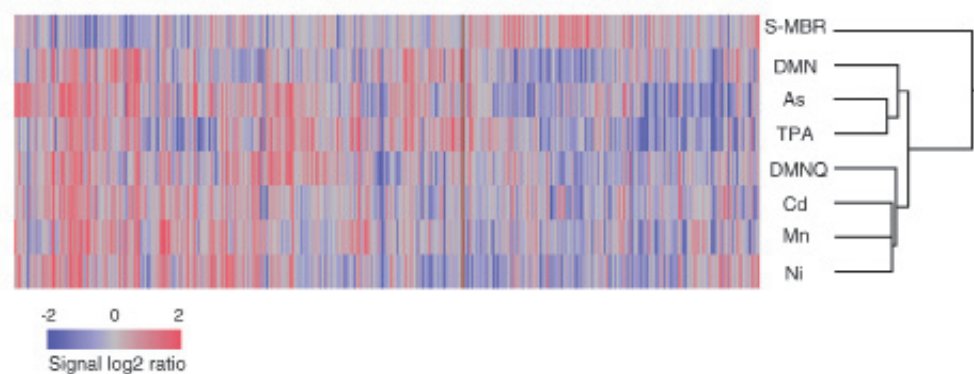


Fig. 4-5 Hierarchical clustering analysis of gene expression profiles of S-MBR effluent and model chemicals

DISCUSSION

Concentration-dependant change of the gene expression responses in human cells exposed to some well-known toxicants has been reported in the previous researches (8-11). Our separate study also revealed concentration-dependant change of the modes of action of inorganic arsenic in HepG2 at various concentrations from 5 nM to 40 μ M (as As_2O_3), and showed the exposure at the sub-lethal concentration (i.e., 90% cell viability) exhibited the various modes of actions including the features stretching into lower or higher concentration regions, while the lowest and highest concentrations had their own unique responses (Chapter 5). Thus, the DNA microarray analysis at the sub-lethal or the maximum concentration, at which more than 80% cell viability was still determined by cytotoxicity test, has been suggested as one reasonable condition to consider the gene expression response particularly when the limited concentrations can be tested. However, this finding had posed a question in the previous **approaches to investigate the gene expression response of the whole wastewater effluents**, where the unequal “exposure does” of effluent constituents could not be avoided due to the day-to-day change of influent load and/or the different performance among the selected processes. In the present study, effluent concentration by RO filtration allowed us to investigate the gene expression response in HepG2 cells exposed to the specific DOC level, which covered more than 80% of dissolved organic matters present in the original effluent. Thus, it was considered that a representative profile of S-MBR effluent at the sub-lethal region was obtained and comparison with that previously obtained for the non-concentrated effluent provided us the insights into the key gene expression response.

Comparison of the S-MBR effluent at IC20 exposure and the selected model toxicants suggested that the impacts of the effluent on gene expression profiles were not well characterized by the selected model chemicals which were supposed to represent heavy metal toxicity, oxidative stress, and carcinogenicity. This is in good agreement with the previous study which demonstrated limited production of reactive oxygen species (ROS) or oxidative stress by the MBR effluents and the secondary treated effluent (12). The gene expression responses against the effluent could be rather characterized by the induction of genes related to “steroid metabolic process” and suppression of those related to “ion homeostasis”, which were overlapped with the biological processes commonly identified in the concentrated and the original effluent. Since the alteration of genes related to lipid metabolism have been reported for various organic contaminants (13-15) and also found in the GO analysis of the selected model chemicals (Supplemental Information C), it was suggested that the overall gene expression profiles or combination of genes from different biological processes were still to identify the feature of the effluent exposure in the transcriptome analysis. Therefore, representativeness of the specific genes and the reproducibility of their expression in the effluent samples are required to be further investigated with the quantitative capability. At present, the genes encoding aldo keto reductase family (e.g., AKR1C2 and AKR1B10) and cytochrome P450 (e.g., CYP1A1 and CYP39A1) are suggested unique to effluent impact.

SUMMARY

Based on their dose-response relationship in exposure of concentrated MBR and AS effluents from 10 to 50 mg-DOC/L, AKR1B10, CYP1A1, GPX2 and GCLM were selected as effluent-responsive marker genes among the commonly altered genes identified in the DNA microarray analysis of two or more effluents. Comparison of gene expression profiles between effluents and model toxicants revealed the uniqueness of the gene expression profiles of the effluents were suggested in comparison to model toxicants (i.e., heavy metals, oxidative stress inducers, and carcinogens). The results altogether suggested RO concentration as an effective pretreatment step for DNA microarray of relatively “clean” effluents.

REFERENCES

- (1) Wilkening, S.; Stahl, F.; Bader, A., Comparison of primary human hepatocytes and hepatoma cell line HepG2 with regard to their biotransformation properties. *Drug Metab Dispos* 2003, 31(8), 1035-1041
- (2) Hoque, A.; Kimura, K.; Miyoshi, T.; Yamato, N.; Watanabe, Y., Characteristics of foulants in air-sparged side-stream tubular membranes used in a municipal wastewater membrane bioreactor. *Sep Purif Technol* 2012, 93, 83-91.
- (3) Hara-Yamamura, H.; Nakashima, K.; Hoque, A.; Miyoshi, T.; Kimura, K.; Watanabe, Y.; Okabe, S. Evaluation of Whole Wastewater Effluent Impacts on HepG2 using DNA microarray-based Transcriptome Analysis, *Env. Sci. Tech.* 2013, 47(10), 5425-5432.
- (4) Kimura, K.; Yamato, N.; Yamamura, H.; Watanabe, Y., Membrane fouling in pilot-scale membrane Bioreactors (MBRs) treating municipal wastewater. *Environ Sci Technol* 2005, 39 (16), 6293-6299.
- (5) Kawata, K.; Osawa, M.; Okabe, S., In Vitro Toxicity of Silver Nanoparticles at Noncytotoxic Doses to HepG2 Human Hepatoma Cells. *Environ Sci Technol* 2009, 43 (15), 6046-6051.
- (6) Kawata, K.; Yokoo, H.; Shimazaki, R.; Okabe, S., Classification of heavy-metal toxicity by human DNA microarray analysis. *Environ Sci & Technol* 2007, 41 (10), 3769-3774.
- (7) Kawata, K.; Shimazaki, R.; Okabe, S., Comparison of gene expression profiles in HepG2 cells exposed to arsenic, cadmium, nickel, and three model carcinogens for investigating the mechanisms of metal carcinogenesis. *Environ Mol Mutagen* 2009, 50 (1), 46-59.
- (8) Gonzalez, H. O.; Hu, J.; Gaworecki, K. M.; Roling, J. A.; Baldwin, W. S.; Gardea-Torresdey, J. L.; Bain, L. J., Dose-responsive gene expression changes in juvenile and adult mummichogs (*Fundulus heteroclitus*) after arsenic exposure. *Marine Environ Res* 2010, 70, 133-141
- (9) Yu, X.; Robinson, J. F.; Gribble, E.; Hong, S. W.; Sidhu, J. S.; Faustman, E. M., Gene expression profiling analysis reveals arsenic-induced cell cycle arrest and apoptosis in p53-proficient and p53-deficient cells through differential gene pathways. *Toxicol App Pharmacol* 2008, 233, 389-403
- (10) Andrew, A. S.; Bernardo, V.; Warnke, L. A.; Davey, J. C.; Hampton, T.; Mason, R. M.; Thorpe, J. E.; Ihnat, M. A.; Hamilton, J. W., Exposure to arsenic at levels found in U. S. drinking water modifies expression in the mouse lung. *Toxicol Sci* 2007, 100(1), 75-87
- (11) Argos, M.; Kibriya, M. G.; Parvez, F.; Jasmine, F.; Rakibuz-Zaman, M.; Ahsan, H., Gene Expression profiles in peripheral lymphocytes by arsenic exposure and skin lesion status in a bangladeshi population. *Cancer Epidemiol Biomarkers Prev* 2006, 15, 1367-1375.
- (12) Fukushima, T.; Hara-Yamamura, H.; Urai, M.; Kasuga, I.; Kurisu, F.; Miyoshi, T.; Kimura, K.; Watanabe, Y.; Okabe, S., Effects of chlorination on toxicity of wastewater effluents from different treatment systems to HepG2 human hepatoblastoma cells. [under review]
- (13) Ratajewski, M.; Walczak-Drzewiecka, A.; Salkowska, A.; Dastych, J., Aflatoxins upregulate CYP3A4 mRNA expression in a process that involves the PXR transcription factor. *Toxicol Lett* 2011, 205 (2), 146-153.
- (14) Castorena-Torres, F.; de Leon, M. B.; Cisneros, B.; Zapata-Perez, O.; Salinas, J. E.; Albores, A., Changes in gene expression induced by polycyclic aromatic hydrocarbons in the human cell lines HepG2 and A549. *Toxicol in Vitro* 2008, 22 (2), 411-421.
- (15) Staal, Y. C. M.; van Herwijnen, M. H. M.; van Schooten, F. J.; van Delft, J. H. M., Modulation of gene expression and DNA adduct formation in HepG2 cells by polycyclic aromatic hydrocarbons with different carcinogenic potencies. *Carcinogenesis* 2006, 27 (3), 646-655.
- (16) Sawada, H.; Takami, K.; Asahi, S., A toxicogenomic approach to drug-induced phospholipidosis: Analysis of its induction mechanism and establishment of a novel in vitro screening system. *Toxicol Sci* 2005, 83 (2), 282-292.
- (24) Wang, J. L., D. P. Yin, et al. (2007). "Dual specificity phosphatase 1/CL100 is a direct transcriptional target of

E217-1 in the apoptotic response to oxidative stress.” *Cancer Res* 67(14): 6737-6744.

5

CHARACTERIZATION OF RESPONSIBLE FRACTION FOR EFFLUENT IMPACTS ON HEPG2 USING MARKER GENES

OBJECTIVES

To search physicochemical properties of biologically active fraction of wastewater effluents, expression levels of the four effluent-responsive marker genes (i.e., CYP1A1, AKR1B10, GPX2, and GCLM) were quantified in HepG2 exposed to physico-chemically fractionated samples.

MATERIALS and METHODS

Cell Culture. Human hepatoma HepG2 cells were provided by Cell Bank, RIKEN BioResource Center (Tsukuba, Japan). HepG2 was selected because it has been frequently used in vitro model for human detoxification of chemicals, is easy to handle, and provides a reproducible human system. However, since it is known that expression of specific genes were extremely low in the cancer derived HepG2, the results could not be simply extrapolated into the normal cells and in vivo (1). Cells were grown in Eagle's minimal essential medium (MEM) (Nissui, Tokyo, Japan), supplemented with 10% fetal bovine serum (FBS) and 60 mg/mL kanamycin. Cells were maintained at 37 °C in the 5% CO₂ humidified incubator.

Sampling site. The sampling was carried out from May to June 2013, at the full-scale municipal wastewater treatment plant (Soseigawa Municipal Wastewater Treatment Plant, Sapporo, Japan), which daily received ca. 130,000 m³ of wastewater via combined sewage system. The plant uses an activated sludge system (AS), which consists of aeration tank and secondary sedimentation basin. Two pilot-scale MBRs were also installed at the plant: the submerged MBR (S-MBR) equipped with hollow-fiber polytetrafluoroethylene (PTFE) membrane with 0.3 µm nominal pore size, and the air-sparged side-stream MBR (AS-MBR) equipped with tubular polyvinylidene difluoride (PVDF) membrane with 0.03 µm nominal pore size (2). The operational conditions and general effluent quality of selected processes are found in elsewhere (3). Grab samples were taken around 10:00 am, from the effluents of the secondary sedimentation basin of the AS and the two MBRs.

RO concentration. Immediately after sampling, the MBR effluents were pre-filtered with 0.45 µm glass fiber filter (Advantec Toyo Kaisha, Ltd., Tokyo, Japan) to remove suspended solids. Concentration of effluent samples was conducted by the cross-flow filtration system for flat-sheet membranes (SEPA CF II Unit; SANKO Ltd., Yokohama, Japan), equipped with polyamide reverse osmosis (RO) membrane (effective surface area: 140 cm²; GE Water & Process Technologies, PA, US) under the constant pressure (2 MPa). The cross-flow speed and permeate flux were 0.1 m/sec and 42 L/m²/h, respectively. During the operation over two nights, the retentate was kept in cold water bath (8 °C), and the salt rejection rate was measured intermittently by the electronic conductivity meter (> 98%). The RO concentrates were filtered with 0.22 µm polyethersulfone membrane using an aseptic vacuum filter system (Corning Japan K.K., Tokyo, Japan) for sterilization, and stored at 4 °C in sterilized muffled bottles until they were subject to fractionation (up to 12 h). The organic content (dissolved organic carbon: DOC) of the samples was measured and adjusted to 50 mg/L by using sterilized Milli-Q water before fractionation.

Fractionation. The Toxicity Identification Evaluation (TIE) study has provided us with an approach to characterize the cause of observed effluent toxicity (4,5), where physical and/or chemical fractionations with biological toxicity testing allow the characterization and identification of key toxicants in various matrices, such as industrial wastewater, sediment, and landfill leachate (6-9). In this study, six manipulations were selected to reduce or eliminate fractions with specific chemical properties in the MBR and AS effluents: 1) aeration with nitrogen (volatile fraction), 2) aeration with pure air (volatile and/or degradable by oxidation), 3) solid phase extraction (SPE) with Sep-Pak C18 (Nihon Waters K.K., Tokyo, Japan) (hydrophobic fraction), 4) SPE with NOBIAS Chelate PA-1 column (Hitachi High-Technologies Corp., Tokyo, Japan) (metals or metal complex), 5) cation exchange with Amberlite IR-120 (sodium form, Aldrich, US), and 6) anion exchange with Amberlite IRA-410 (chloride form, Aldrich). In each manipulation, 35 mL (from two aeration) or 40 mL (from SPE and ion exchange) of treated samples were obtained and again sterilized with 0.22 µm filter.

Treatment. Prior to exposure, 8.7 mL of samples before and after manipulations was combined with 1 mL of $\times 10$ concentrated MEM, and supplemented with 1% fetal bovine serum (FBS) and 60 mg/mL kanamycin. Finally, pH was adjusted to 7.2-7.5 by adding 7.5% sodium hydrogen carbonate solution. Cells were seeded onto culture vessels in 105 cell/mL and incubated overnight to grow 40-50% confluent. The normal culture medium was replaced by the prepared samples and incubated for 48 h.

Cytotoxicity test. Cell viability of HepG2 after 48 hour was studied based on the mitochondrial dehydrogenase activity determined by MTT, which measured the amount of formazane resulted from reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT). MTT (Sigma-Aldrich, Japan) dissolved in PBS was added onto the treated cells seeded in 96-well plates and incubated for 4 hours. Supernatant was removed after centrifugation for 5 min at 3000 rpm, and the amount of formazane pigment in DMSO (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) elutes was measured at 540 nm by plate reader (multilabel counter ARVO MX, PerkinElmer, MA, US).

Quantitative Real-Time Reverse-Transcriptase PCR (qPCR). The expression levels of selected genes were quantified using the quantitative real-time reverse-transcriptase PCR (qPCR) assay. Single-strand cDNA was synthesized from 25 ng/ μ L total RNA in reaction cocktail of PrimeScript RT reagent Kit (Takara, Shiga, Japan). The forward and reverse primers for selected genes and endogenous reference were designed based on the previous studies (Chapter 4). The qPCR was carried out with ABI prism 7500 (Applied Biosystems, Foster City, CA) using SYBR Ex Taq II (Tli RNaseH Plus) (Takara, Shiga, Japan), according to manufacturer's instruction. The amplification reaction consisted of initial incubation at 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 60 s. Data were analyzed by the relative quantification method using the Ct values normalized with the endogenous reference, GAPDH, which was measured in all runs. The fold induction/suppression of each target gene was calculated by dividing the normalized Ct value in a sample with that of the control. All assays were tested for presence of unspecific amplicons and primer dimmers by melting curve analysis.

Chemical analysis. DOC was measured by TOC analyzer (Shimadzu Corp., Kyoto, Japan), while concentration of nitrogen species (i.e., NO₃⁻, NO₂⁻, and NH₄⁺) were determined by ion chromatography. The electrical conductivity (EC) and pH were measured on an EC meter (CM-31P, Tokyo, DKK-TOA Corp., Japan) and a pH/ion meter (HM-30P, DKK-TOA Corp.), respectively. UV absorbance measurement, which is an indicator of the humic faction (10), was performed on spectrometer at 254 nm (V-630 BIO, JASCO, Tokyo, Japan). Detail analysis of organic and inorganic contents of the effluents was further conducted by ICP-AES, and LC-OCD. Concentrations of eight heavy metals (i.e., Al, As, Cd, Cr, Cu, Mn, Pb and Zn) were analyzed using the Inductively Coupled Plasma-optical Emission Spectrometer (ICP-AES) (ICPE-9000, Shimadzu Corp.).

Dissolved organic matter was characterized by size exclusion chromatography with a liquid chromatography with online organic carbon detection (LC-OCD) (11,12). The LC-OCD system (DOC-LABOR) was equipped with a size exclusion chromatography column HW-50S filled with Toyopearl resin (pore size of 125 Å). Three detectors were installed in series in a sequence of UV detector (UVD), organic carbon detector (OCD) and organic nitrogen detector (OND). The LC-OCD enables the quantification (mgC/L) of organic matter fractions such as biopolymers (BP; total proteins and polysaccharides content without distinction), humic substances (HS), buildings blocks (BB; humic-like material of lower molecular weight), low molecular weight acids and low molecular weight neutral substances (LMW).

RESULTS and DISCUSSION

Cytotoxicity before and after manipulations in TRE. Fig. 5-1 shows viability of HepG2 exposed to the concentrated effluents before and after six different manipulations (i.e., aeration with nitrogen, aeration with pure air, SPE with C18, chelating, cation exchange, anion exchange). The result clearly demonstrated that the effluent both before and after manipulations were not cytotoxic. Significant increase of the cell viability after three manipulations (i.e., aeration with pure air, C18, and chelating) in AS effluent might be due to the generation of more biologically active components by oxidation or removal of masking agents by C18 and chelating resin. At present, however, it is difficult to tell if such increase of cell viability in cytotoxicity test represents innocuous or hazardous impacts.

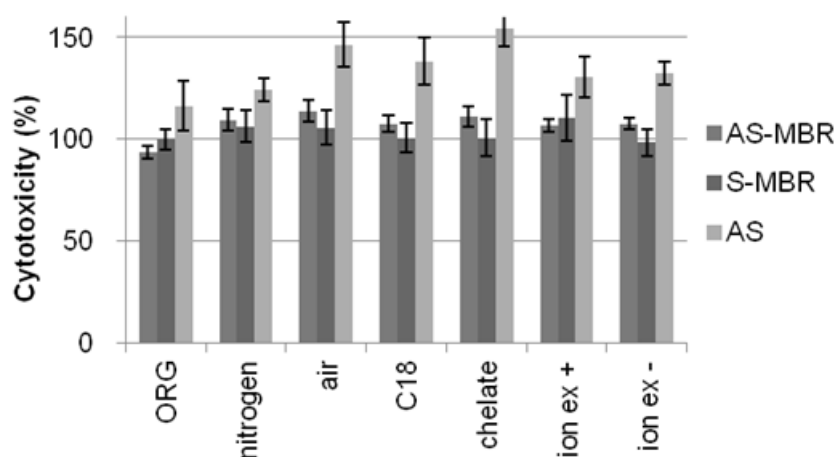


Fig. 5-1 Cytotoxicity before and after manipulations. (DOC before manipulation: 50 mg/L)

Gene expression levels before and after manipulation. The expression levels of the selected marker genes, which represented effluent impacts, were measured for before and after manipulations by qPCR assay (Fig. 5-2). Although there was no significant change in FCs of AKR1B10 and GCLM before and after manipulations, the relative expression level of CYP1A1 dropped after C18 and chelating, and slightly decreased after anion exchange. Similar trend was also observed for GPX2, particularly in the cells exposed to S-MBR effluent. These results suggested that the selected marker genes represent the effluent fractions with different chemical properties. CYP1A1 and GPX2 responded to the hydrophobic fraction and/or those with anionic feature since their expression was significantly reduced after SPE with C18 and chelating and anion exchange. Whereas, it was hypothesized that AKR1B10 and GCLM responded to the neutral and hydrophilic fraction which was not be able to remove by any of the selected manipulations, or they are non-specific markers. The qPCR data was in good agreement with the DOC removal calculated for C18, chelating resin and anion exchange resin (Table 5-1). Interestingly, chelating with lower DOC removal (ca. 20%) than C18 (>50%) were more effective to mitigate the induction of CYP1A1 and GPX2. Thus, it was more likely that responsible organic fraction formed a complex with metals.

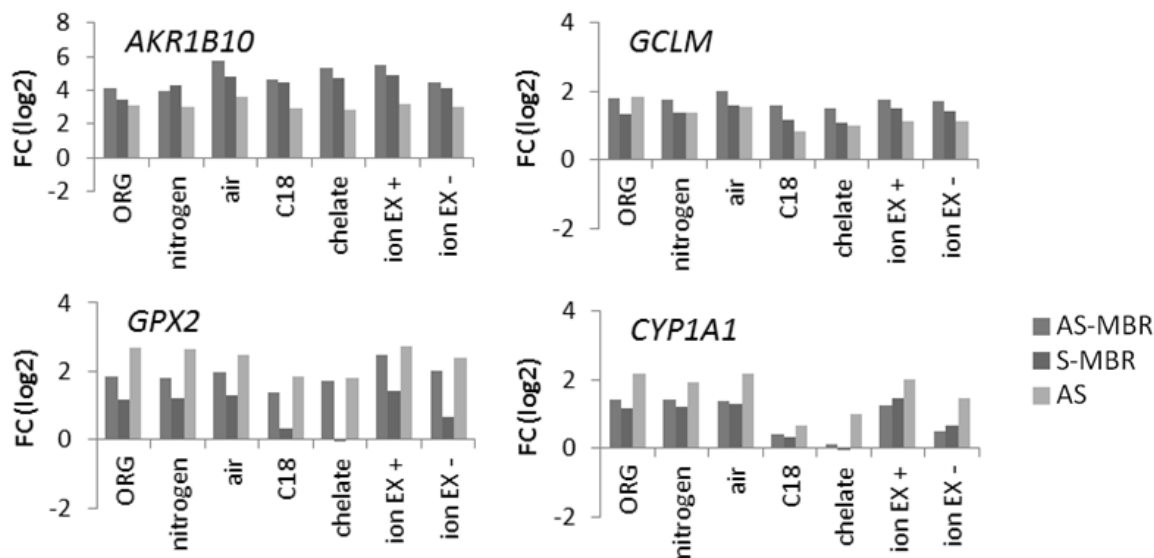


Fig. 5-2 Expression levels of four selected marker genes before and after manipulations.
(DOC before manipulation: 50 mg/L)

Cytotoxicity in various size fractions. Fig. 5-3 shows viability of HepG2 exposed to the four different size fractions (i.e., F1: less than 1,000 Da; F2: 1,000 Da-3,500 Da; F3: 3,500-10,000 Da; F4: 10,000 Da-0.2 μ m). The result also showed that none of the size fractions had apparent cytotoxicity.

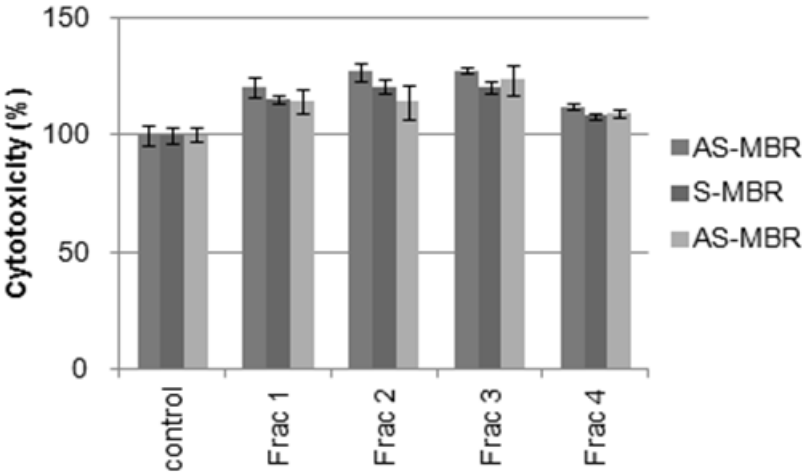


Fig.5-3 Cytotoxicity of size-fractionated samples (DOC: 5 mg/L)

Gene expression level in various size fractions. The expression levels of the selected marker genes were measured for the four different size fractions by qPCR assay (**Fig. 5-4**). Since DOC of all samples were adjusted to their lowest DOC, exposure concentration was set on 5 mg/L, thereby the range of fluctuation was relatively small ($FC < 3$). For AKR1B10, GCLM and GPX2, there was no significant change between the size fractions, perhaps because their responsible fraction were not size specific, or more likely, the range of fluctuation of these marker genes were much smaller than CYP1A1. Significant induction of CYP1A1 was observed F1 (less than 1,000 Da) and F3 (3,500-10,000 Da). The dominance of F1 in total DOC taken into consideration (**Table 5-2**), it was indicated that the dissolved matters with the molecular size 1,000 Da or smaller had a largest responsibility for CYP1A1 induction, and with colloidal part from less than 10,000 had the second.

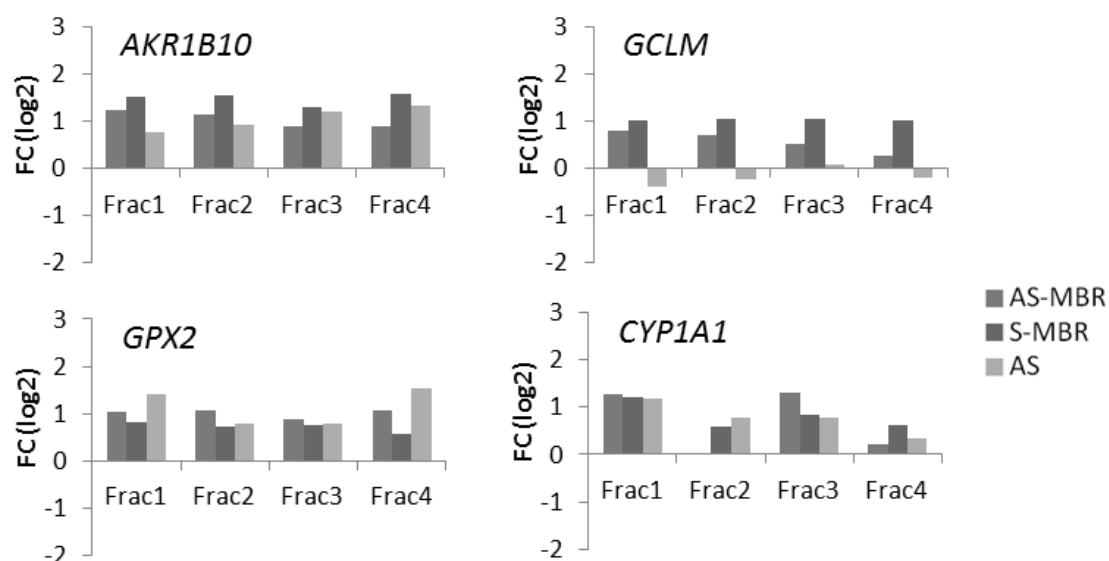


Fig. 5-4 Expression levels of size-fractionated samples (DOC: 5 mg/L)

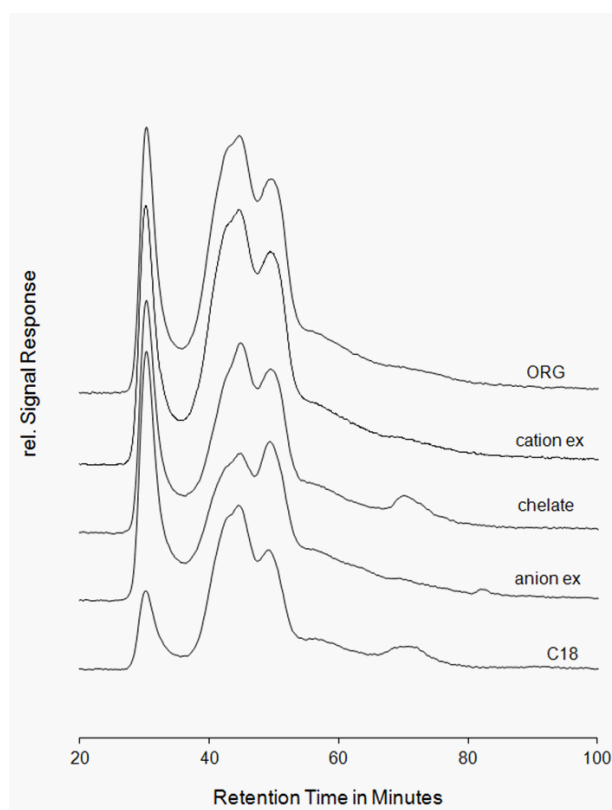
Change of water quality. Organic content and UV adsorption at 254 nm before and after manipulation are summarized in Table 5-1. Significant change of DOC and UV adsorbance were not observed after aerations and cation exchange, while SPE with C18, chelation and anion exchange reduced DOC and UV adsorbance by 20-57% and 30-66%, respectively. Higher removal rates of UV adsorbance than those of DOC after chelation and anion exchange suggested the organic content rich in saturated bonds such as humic substances (HS) were preferably captured by the manipulations. It was speculated that humic acids with disassociated hydroxylic and carboxylic groups were efficiently removed by anion exchange, and those HS forming metal complex may be contained in the fraction removed by chelation. Table 5-2 shows the organic content and UV adsorbance of each size fraction. In common among the effluents, the fraction 4 with the largest particle size (i.e., 10,000 Da < size < 0.2 μ m) accounted for the largest percentage (i.e., 43-52%) of the total organic content in the concentrated effluents, followed by fraction 1 with the smallest particle size less than 1,000 Da. The ratio of UV adsorption to DOC (SUVA values as an indicator for HS) suggested that HS of S-MBR effluent mostly appeared in the size range less than 1,000 Da.

Table 5-1 Organic content and UV adsorbance at 254 nm before and after manipulation.

manipulation	DOC (mg/L) (% removal)			UV 254 (1/cm) (% removal)		
	AS-MBRS-MBR	AS		AS-MBRS-MBR	AS	
Original effluent	50.9	44.1	50.0	1.09	0.83	0.89
Aeration with nitrogen	49.6	43.3	49.8	1.14	0.86	1.01
Aeration with pure air	49.9	43.8	49.3	1.15	0.77	0.93
Solid phase extraction (C18)	23.0(55)	18.8(57)	25.0(50)	0.48(56)	0.28(66)	0.43(52)
Chelation	39.5(22)	33.7(24)	40.0(20)	0.69(37)	0.43(48)	0.59(34)
Cation exchange	49.9(2)	42.7(3)	47.0(6)	1.16(-6)	0.75(10)	0.95(-7)
Anion exchange	39.1(23)	30.3(31)	37.0(26)	0.76(30)	0.48(42)	0.59(34)

Table 5-2 Organic content and UV adsorbance at 254 nm of size fractionated samples.

Fraction	Target size	DOC (mg/L) (% in total)			UV 254 (1/cm) (% in total)	
		AS-MBRS-MBR	AS		AS-MBRS-MBR	AS
1	< 1,000 Da	11.1(32)	8.2(18)	9.0(21)	3.37(36)	4.37(34)
2	1,000 Da < size <3,500 Da	4.3(12)	6.8(15)	6.3(15)	0.98(10)	1.52(12)
3	3,500 Da <size <10,000 Da	4.4(13)	7.1(15)	7.4(17)	0.89(10)	1.44(11)
4	10,000 Da <size <0.2 μ m	14.7(43)	24.3(52)	20.5(47)	4.10(44)	5.70(44)

**Fig. 5-5** Expression levels of size-fractionated samples (DOC: 5 mg/L)

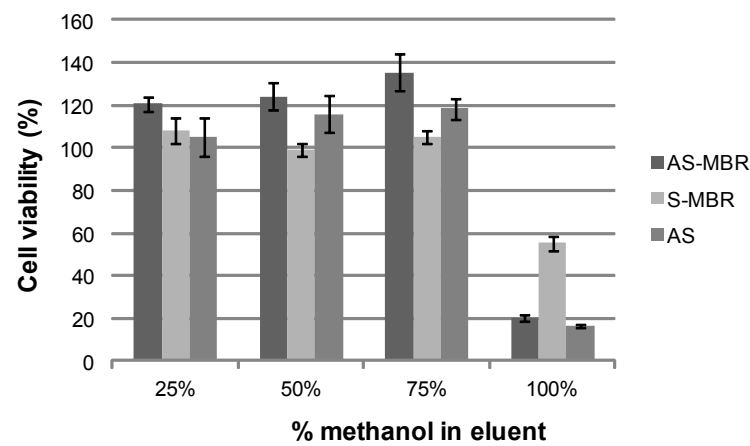


Fig. 5-6 Cell viability of HepG2 exposed to methanol elutes from C18 column

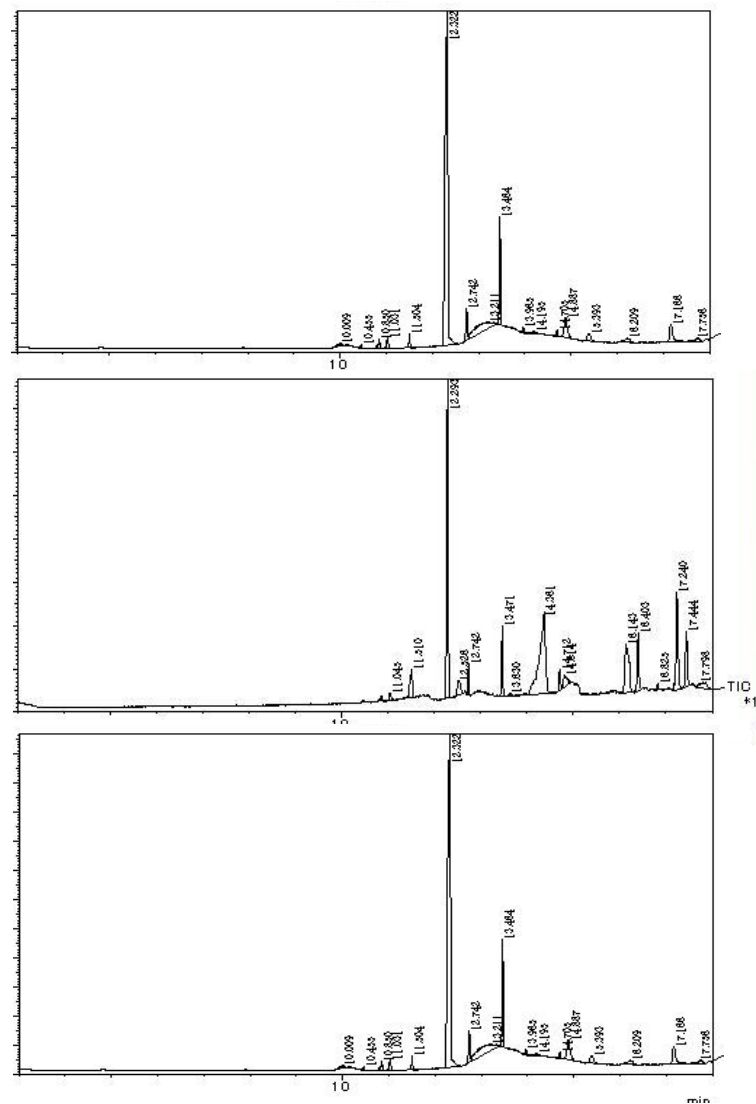


Fig. 5-7 GC/MS chromatogram of 100% methanol elutes from C18 column

CONCLUSIONS

Quantification of four selected marker genes (i.e., AKR1B10, CYP1A1, GPX2 and GCLM) before and after various manipulations suggested that hydrophobic organic content, which may be forming complex with metals, were responsible fraction for a part of gene expression response observed in HepG2 exposed to MBR and AS effluents (particularly CYP1A1-inducible fraction). The chemical analysis indicated that the responsible fraction would behave together with humic substances or their building blocks with fell in a size range from 300 to thousands of Da.

REFERENCES

- (1) Wilkening, S.; Stahl, F.; Bader, A., Comparison of primary human hepatocytes and hepatoma cell line HepG2 with regard to their biotransformation properties. *Drug Metab Dispos* 2003, 31(8), 1035-1041
- (2) Hoque, A.; Kimura, K.; Miyoshi, T.; Yamato, N.; Watanabe, Y., Characteristics of foulants in air-sparged side-stream tubular membranes used in a municipal wastewater membrane bioreactor. *Sep Purif Technol* 2012, 93, 83-91.
- (3) Hara-Yamamura, H.; Nakashima, K.; Hoque, A.; Miyoshi, T.; Kimura, K.; Watanabe, Y.; Okabe, S. Evaluation of Whole Wastewater Effluent Impacts on HepG2 using DNA microarray-based Transcriptome Analysis, *Env. Sci. Tech.* 2013, 47(10), 5425-5432.
- (4) United States Environmental Protection Agency (US-EPA). *Methods for Aquatic Toxicity Identification Evaluations - Phase I Toxicity Characterization Procedures*, 2nd, ed.; Office of Research and Development, Washington, DC, 1991
- (5) United States Environmental Protection Agency (US-EPA). *Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants*; Office of Research and Development, Washington, DC, 1999
- (6) Erten-Unal, M.; Gelderloos, A. B.; Hughes, J. S., A toxicity reduction evaluation for an oily waste treatment plant exhibiting episodic effluent toxicity. *Sci Tot Environ* 1998, 218, 141-152.
- (7) Jo, H.; Park, E.; Cho, K.; Kim, E.; Jung, J., Toxicity identification and reduction of wastewaters from a pigment manufacturing factory 2008, *Chemosphere*, 70, 949-957.
- (8) Krantzberg, G.; Boyd, D., The biological significance of contaminants in sediments from hamilton harbour, Lake Ontario. *Environ Toxicol Chem* 1992, 11(11), 1527-1540.
- (9) Jemec, A.; Tisler, T.; Zgajnar-Gotvajn, A., Assessment of landfill leachate toxicity reduction after biological treatment. *Arch Environ Contam Toxicol* 2012, 62(2), 210-21.
- (10) Chen, Z.; Valentine, R. L., Formation of N-Nitrosodimethylamine (NDMA) from Humic Substances in natural water. *Environ Sci Technol* 2007, 41(17), 6059-6065,
- (11) Huber, S. A.; Balz, A.; Abert, M.; Pronk, W., Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography-organic carbon detection-organic nitrogen detection (LC-OCD-OND). *Water Res* 2011, 45(2), 879-885.
- (12) Yamamura, H.; Kimura, K.; Watanabe, Y., Effects of NOM size on reversible and irreversible membrane fouling in microfiltration of surface water [in preparation]

6

CONCENTRATION-DEPENDENT CHANGES IN MODES OF TOXIC ACTIONS OF INORGANIC ARSENIC

OBJECTIVE

As an example of model toxicants, the DNA microarray analysis was applied to human hepatocarcinoma cell lines (HepG2) exposed to arsenic trioxide at various concentrations to better understand the relationship between gene expression response and molecular mechanism of toxicity. HepG2 cells were exposed to a wide range of arsenic trioxide concentrations from environmentally relevant level to apparent cytotoxic level (i.e., As_2O_3 ; 5 nM to 40 μM). The potential marker genes of arsenic exposure were proposed in this study.

MATERIALS and METHODS

Cell Culture and treatments. Human hepatoma HepG2 cells were provided by Cell Bank, RIKEN BioResource Center (Tsukuba, Japan). Cells were grown in Eagle's minimal essential medium (MEM) (Nissui, Tokyo, Japan), supplemented with 10% fetal bovine serum (FBS) and 60 mg/mL kanamycin. Cells were maintained at 37°C in the 5% CO_2 humidified incubator. For experimental use, cells were seeded into culture vessels and pre-incubated overnight. Chemical treatments were done by replacing the culture medium with normal medium (as a control) and that containing arsenic trioxide (As_2O_3) or ϵ -caprolactam at various concentrations. Since ϵ -caprolactam is the only chemical categorized in IARC's group 4 (Probably Not Carcinogenic), the cellular response to ϵ -caprolactam exposure was considered to have the least association with carcinogenic actions.

Neutral red dye uptake assay (NRU assay). Cytotoxicity of inorganic arsenic on HepG2 was determined by using the neutral red dye uptake assay (NRU assay). Briefly, cells were seeded in 96-well plates and treated with arsenic trioxide (As_2O_3) at 12 different concentrations (0.001–40 μM) and ϵ -caprolactam at 10 different concentrations (0.1–40 mM), respectively. After 48 h exposure, the medium was replaced with medium containing 0.005% neutral red dye and incubated for 2 h. Optical density of the eluted dye in extracting solution (50% ethanol, 49% Milli-Q water and 1% acetic acid) was then measured at 540 nm by a plate reader (ARVO MX, PerkinElmer, US).

Measurement of ROS generation. Previous studies reported that ROS generated by arsenic exposure damage lipids, protein and DNA, induce cell proliferation and apoptosis, and associate with carcinogenesis (13, 14). ROS generation was measured by OxiSelect™ ROS Assay Kit (Cosmo Bio Co., Ltd., Tokyo, Japan) based on the manufacturer's instruction. Briefly, cells were seeded and treated in a similar manner to NRU assay as described above, except the medium was first replaced with the medium containing 1 mM the 2',7'-dichlorofluorescein-diacetate (DCFH-DA) for 1 h pre-incubation and then changed to the medium containing chemicals for 30 min exposure. Cells were incubated in dark. After 30 min, fluorescence was measured by the plate reader (excitation at 488 nm and emission at 530 nm).

RNA extraction and DNA microarray. The DNA microarray-based transcriptome analysis has been used as a comprehensive, rapid and sensitive tool to detect the genome-wide gene expressions, whose patterns represent the primary interactions between the constituents of effluents and biota, and widely applied for environmental pollutants (15,16,17,18). After cell treatment, cells were lysed on 60 mm culture dishes, and total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany). For replication, **three dishes were prepared for each sample and individually treated in parallel**. Total RNAs were quantified using the Nanoview (GE Healthcare, US). The integrity of the RNA samples was also checked by microchip electrophoresis using the Cosmo Eye with the i-RNA Kit (Hitachi Chemical, Tokyo, Japan). RNA with a A260/A280 ratio 1.8 or higher was used for DNA microarray analysis with Human Genome Focus Array (Affymetrix, Santa Clara, CA). Target preparation and hybridization were carried out as described in the manufacturer's instruction. After hybridization, the array was washed, stained with streptavidin-phycoerythrin using the Affymetrix GeneChip Fluidics Workstation 450, and scanned on a GeneArray™ scanner (Affymetrix). The raw data files [cell intensity (CEL) files] were created from scanned images by GCOS (GeneChip Operating Software, Affymetrix). The files are available on Gene

Expression Omnibus (GEO; <http://www.ncbi.nlm.gov/geo>) of the National Center for Biotechnology Information (NCBI) (accession number: under application for approval).

Quantitative real-time reverse-transcriptase PCR (qPCR). The expression levels of selected genes were quantified using the quantitative real-time PCR (qPCR). RNA was extracted in the same manner as described earlier. Single-strand cDNA was synthesized with PrimeScript RT reagent Kit (Takara, Shiga, Japan). The forward and reverse primers for selected genes and endogenous reference were designed based on the previous studies: pituitary tumor-transforming 1 (PTTG1), Forward: 5-AACTGCAAGAAAGGCTTTGG-3, Reverse: 5-CAAACAGGTGGCAATTCAAC-3 (19); cell division cycle 25 homolog B (CDC25B), Forward: 5-CTCATAGTGCCCCACTGGT -3, Reverse: 5-GTGGTCACTGTC-CAGGAGGT-3 (20); ubiquitin-conjugating enzyme E2C (UBE2C), Forward: 5-GGATTCTGCCTTCCCTGAA -3, Reverse: 5-GATAGCAGGGCGTGAGGAAC-3 (21), and glyceraldehydes-3-phosphate dehydrogenase (GAPDH), Forward: 5-TTGGTATCGTGGAAGGACTCA-3, Reverse: 5-TGTCATCATATTTGGCAGGTT-3 (17). The qPCR was performed using ABI Prism 7000 (Applied Biosystems, Foster City, CA) with a SYBR Premix Ex Taq (Takara, Shiga, Japan). For each sample, the cycle time (Ct) values of the target genes were first normalized with those of the endogenous reference (GAPDH) by using the calibration curves obtained by serial dilution of total RNA from untreated cells. Then, relative differences between control and treatment groups were calculated and expressed as fold changes to the controls.

Statistics. For microarray analysis, probe-level data in CEL files were processed and analyzed using the Avadis 4.3 prophetic (Strand Genomics, Redwood City, CA). Data were normalized using MAS5 algorithm, and the features which were NOT flagged “Present” in all three replicates were rejected. We identified differentially expressed genes based on the two criteria: 1) a t-test p-value threshold of 0.05 and 2) a minimum fold change of 2 between the controls and exposed data sets. Gene ontology (GO) assignments and clustering into functional groups were performed using a web-based GO search engine, FatiGO available from Babelomics (<http://babelomics.bioinfo.cipf.es/>).

RESULTS

Cytotoxicity of inorganic arsenic. To obtain the basic information on concentration-dependent cytotoxicity of inorganic arsenic, the cell viability was measured after 48 hour exposure at 12 different concentrations from 0.001 μM to 40 μM by using the NRU assay (**Fig. 5-1**). The cell viability was 111% at 0.001 μM and increased up to 135% as the concentration increased to 0.5 μM . Decline of cell growth was observed at 6 μM and higher. The severest exposure of arsenic in this study (i.e., 40 μM) suppressed the cell viability down to 10%. Based on these results, five different concentrations were selected for ROS measurement and DNA microarray experiments: 0.005 μM (environmentally relevant concentration), 0.07 μM (equivalent to Japanese drinking water standard), 0.5 μM (concentration with the highest viability: 135%), 6 μM (sub-lethal concentration with cell viability of 90%), and 40 μM (concentration with the lowest cell viability: 10%).

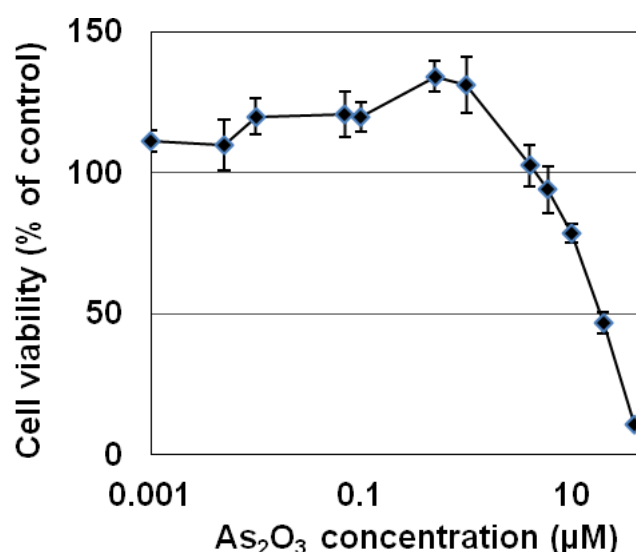


Fig. 5-1 Concentration-dependent change in cytotoxicity of As₂O₃. Cell viability of HepG2 was measured after 48 h exposure to As₂O₃ by NRU assay. Data represent the mean $\pm$ SD of eight replicates.

ROS generation by arsenic exposure. The ROS generation in HepG2 after exposure to five different arsenic concentrations was measured (Fig. 5-2). Significant generation of the total ROS was observed at the concentrations 0.07 μ M or higher. The highest ROS was generated at 0.5 μ M and 6 μ M (>160%), which were even higher than that produced by the positive control, 10 μ M H₂O₂. The ROS generation fell down to 130% at 40 μ M exposure probably due to the low cell viability (Fig. 5-1).

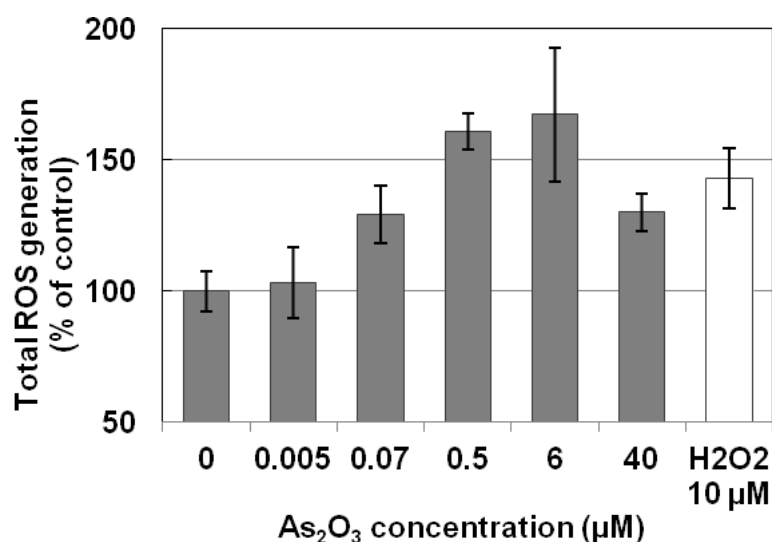


Fig. 5-2 ROS generation in HepG2 exposed to As₂O₃ and 10 μ M H₂O₂. Significant generation of ROS was observed at the concentrations 0.07 μ M or higher. Data represent the mean $\pm$ SD of eight replicates.

Gene expression profiles after arsenic exposures. The DNA microarray was used to obtain the global gene expression profile of HepG2 exposed to various concentrations of inorganic arsenic. The number of differentially expressed genes increased from 55 to 1216 as the concentration increased from 0.005 μM to 40 μM (**Table 5-1**). Comprehensive list of differentially expressed genes for arsenic and ϵ -caprolactam exposures can be found in Supplemental Information (S7). The suppressed genes accounted for more than half of the differentially expressed genes at all concentrations except for 0.07 μM , where the induced genes were more dominant (i.e., 64%).

Table 5-1 A summary of differentially expressed genes in HepG2 exposed to As_2O_3 , which was compared with those exposed to ϵ -caprolactam, TPA, and TCE.

Chemicals	Induced	Repressed	Total
As_2O_3 0.005 μM	24	34	55
0.07 μM	110	62	172
0.5 μM	98	143	241
6 μM	225	340	565
40 μM	565	651	1216
Caprolactam 15 mM	78	81	159
TPA 0.1 μM	313	342	655
TCE 2 mM	197	248	445

Functional analysis of differentially expressed genes using FatiGO identified 56, 133, 187, 270, and 318 biological processes (level 6) in arsenic exposures at 0.005, 0.07, 0.5, 6, and 40 μM , respectively. The percentage of the categorized genes to the total altered genes is shown for 11 major biological processes (**Fig. 5-3a**): RNA biosynthetic process; phosphorylation; M phase; microtubule-based process; intracellular transport; regulation of nucleobase, nucleoside, nucleotide and nucleic acid; cell death; proteolysis; steroid metabolic process; lipid biosynthetic process; regulation of progression through cell cycle. The cell cycle genes, which were found in “M phase”, “microtubule-based process”, and “regulation progression through cell cycle”, were expressed in a different manner between low and high arsenic exposures. In 0.07-6 μM exposure, the genes categorized in “M phase” and “microtubule-based process” were induced. Particularly, CDC20 playing a role in the control of checkpoint in cell cycle and CDC25B encoding the protein necessary for entry into mitosis were over expressed commonly in 0.07, 0.5, and 6 μM exposures. The induction of UBE2C, BIRC5, BUB1B, CKS1, and CKS2 was observed at 0.5 and 6 μM exposures. However, some cell cycle genes such as CDC2, CCNA2, and CCNB2 were suppressed in 40 μM exposure.

Although clear concentration-dependent changes were not observed at the biological process level, individual genes related to “stress response”, “cell cycle arrest” and “induction of apoptosis” were altered to a larger extent (> 50-fold in 2-base logarithm) in the high exposures (**Table 5-2**). Stress response genes, such as heat-shock protein (HSPA1A, HSPA1B, HSPA4L, HSPB1, HSPH1), metalotionaine (MT1H, MT1X, MT2A) and oxidative stress (HMOX1), were significantly induced at 6 μM and 40 μM . Genes related to a major antioxidant, glutathione, were also induced (GPX3, GSR, GSTM3, GSTO1, and GSS). In 40 μM exposure, induction of cell cycle arrest genes (CDKN1A and CDKN3) and apoptotic genes (CASP4, CASP7, CASP8, FAS, GADD45A, and GADD45B) were significant. The suppression of the genes involved in DNA repair (i.e., RAD23B, CETN2, CETN3) was also observed from low concentrations (0.07 μM or higher), and most significant in 40 μM (i.e., NTHL1, APE1, MUTYH).

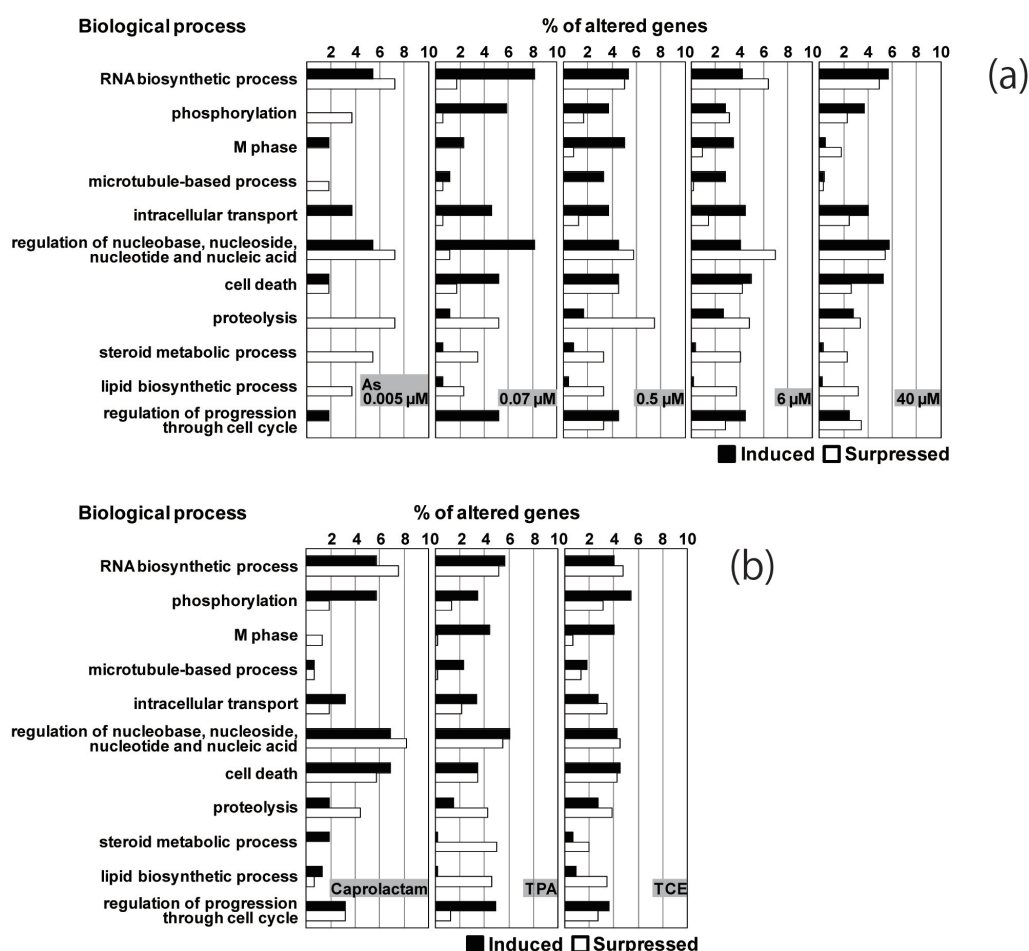


Fig. 5-3 Gene Ontology (GO) of differentially expressed genes in exposure of arsenic (a) and model chemicals (b). GO assignments and clustering into functional groups were performed using a web-based GO search engine, FatiGO available from Babelomics (<http://babelomics.bioinfo.cipf.es/>).

Gene expression profiles of arsenic, ϵ -caprolactam, TPA and TCE. The gene expression profile of arsenic exposure was analyzed and compared with those of two well-known carcinogens, 12-O-tetradecanoylphorbol-13-acetate (TPA) and tetrachloroethylene (TCE) and a non carcinogenic compound, ϵ -caprolactam (CAP). Cytotoxicity of ϵ -caprolactam appeared only at the several tens mM level (data not shown). The sub-lethal concentrations, where the cell viability was between 70-90%, were used for gene expression analysis except for the carcinogen promoter (TPA). The TPA concentration marked significant cell growth (i.e., 0.1 μM) was chosen. The more detailed information on gene expression profiles of TPA and TCE is available elsewhere (17).

Exposure of CAP, TPA, and TCE to HepG2 cells significantly altered 163, 655, and 445 genes (Table 5-1), which were further categorized into 186, 243, and 259 biological processes (level 6), respectively, by functional analysis. The percentage of the categorized genes to the total altered genes is shown for major biological processes (Fig. 5-3b). To be noted, the genes categorized in “M phase” were significantly induced by both TPA and TCE but not by ϵ -caprolactam. In addition, the genes related to “steroid metabolic process” and “lipid biosynthetic process” were suppressed by only TPA and TCE exposures. Those gene expression changes observed for model carcinogens were also found in the inorganic arsenic exposure. It has been reported that the suppression of lipid biosynthetic process was triggered by p38 MAPK pathway (22), which could be activated by arsenic and TPA (23, 24).

Table 5-2 Fold changes of genes related to stress response, apoptosis and cell cycle arrest (the positive: up-regulation, the negative: down-regulation).

	Gene symbol	Gene name	log2 Fold Change				
			Arsenic concentration (μM)				
			0.005	0.07	0.5	6	40
Stress response genes	<i>HSPA1A</i>	heat shock 70kDa protein 1A	-	2.04	-	4.18	17.32
	<i>HSPA1B</i>	heat shock 70kDa protein 1B	-	-	-1.15	-	7.32
	<i>HSPA4L</i>	heat shock 70kDa protein 4-like	-	-	-	2.41	2.16
	<i>HSPB1</i>	heat shock 27kDa protein 1	1.62	2.17	2.54	5.76	4.41
	<i>HSPH1</i>	heat shock 105kDa/110kDa protein 1	-	1.95	1.63	4.46	5.21
	<i>MT1H</i>	metallothionein 1H	-1.77	-1.65	-1.24	6.26	5.86
	<i>MT1X</i>	metallothionein 1X	-1.30	-1.18	-1.20	9.99	13.58
	<i>MT2A</i>	metallothionein 2A	-	-	-	6.51	5.52
	<i>HMOX1</i>	heme oxygenase (decycling) 1	-	-	7.92	66.94	59.00
	<i>GPX3</i>	glutathione peroxidase 3 (plasma)	-	2.03	2.48	-	2.21
	<i>GSR</i>	glutathione reductase	-	1.38	1.67	3.07	1.97
	<i>GSTM3</i>	glutathione S-transferase M3 (brain)	-1.40	-	-	1.88	3.30
	<i>GSTO1</i>	glutathione S-transferase omega 1	-	-	1.42	2.08	2.16
	<i>GSS</i>	glutathione synthetase	-	-	1.22	1.32	2.02
Apoptotic genes	<i>CASP4</i>	caspase 4, apoptosis-related cysteine peptidase	-	-2.17	-	-	2.38
	<i>CASP7</i>	caspase 7, apoptosis-related cysteine peptidase	1.36	-	-	-	2.47
	<i>CASP8</i>	caspase 8, apoptosis-related cysteine peptidase	-	-1.59	-	1.38	2.01
	<i>FAS</i>	Fas (TNF receptor superfamily, member 6)	-	-	-	-	2.55
	<i>GADD45A</i>	growth arrest and DNA-damage-inducible, alpha	-	-	-	-	2.84
	<i>GADD45B</i>	growth arrest and DNA-damage-inducible, beta	-	-	1.82	2.84	22.61
Cell cycle arrest genes	<i>CDKN1A</i>	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	-	-	-	2.94	10.19
	<i>CDKN3</i>	cyclin-dependent kinase inhibitor 3	-	-	2.12	-1.65	2.06

Table 5-3 Differentially expressed genes categorized in “M phase” compared between arsenic and model carcinogen exposures.

	Induced	Suppressed
As₂O₃ 0.005 μM	<i>MAD2L1</i>	
0.07 μM	<i>CDC20, CDC25B, DCTN1, PLK1</i>	
0.5 μM	<i>AURKB, BUB1, BUB1B, CALR, CDC20, CDC25B, CKS2, KIF2C, NUSAP1, PTTG1, TPX2, UBE2C,</i>	<i>CCNG2, NEK4</i>
6 μM	<i>AURKA, BIRC5, BUB1, BUB1B, CALR, CCNA2, CCNB2, CDC20, CDC25B, CKS2, KIF23, KIF2C, MPHOSPH1, NCAPG, NUSAP1, PLK1, PTTG1, SPAG5, TPX2, UBE2C</i>	<i>CETN2, CETN3, FBXO5, NEDD9, NEK4</i>
40 μM	<i>ANAPC10, DCTN1, MYH9, NUDC, PSMD13, SHC1</i>	<i>ANAPC1, BIRC5, CALR, CCNA2, CCNB2, CDC2, CDC25C, CETN3, EML4, MAD1L1, MAD2L1, NCAPG, NEDD9, PBK, PLK1, RAD50, RUVBL1, SC65, UBE2C, YEATS4, ZWINT</i>
TPA	<i>AURKA, BIRC5, BUB1B, CCNA2, CCNB2, CDC2, CDC20, CDC25B, CKS2, DUSP13, KIF23, KIF2C, MAD1L1, MAD2L1, MPHOSPH1, NCAPG, NEK2, NOLC1, NUSAP1, PBK, PLK1, PTTG1, RPA1, SHC1, SMC4, SPAG5, TPX2, UBE2C, ZWINT</i>	<i>CALR</i>
TCE	<i>AURKA, BIRC5, CCNB2, CDC2, CDC25B, CKS2, KIF2C, MAPRE2, NCAPG, NEK2, NUSAP1, PBK, PTTG1, RAD50, SPAG5, SHC1, TPX2, UBE2C</i>	<i>CRYAA, SNHG3-RCC1, RCC1</i>

Quantification of selected gene expressions. Among the differentially expressed genes identified in the DNA microarray experiment, CDC25B, UBE2C, and PTTG1 were selected as marker genes for the pro-carcinogenic action of inorganic arsenic at low concentrations. Their relative expression levels to the control are shown in **Fig. 5-4**. The expression of CDC25B increased by 2-fold as the arsenic concentration increased up to 6 μM , and thereafter the gene expression decreased due to the cell death. Similarly, UBE2C exhibited a concentration-dependent increase of the expression level up to 3.4-fold at 6 μM , followed by a sharp decline. The expression of PTTG1 was only significant in the moderate concentrations (i.e., 6 and 10 μM).

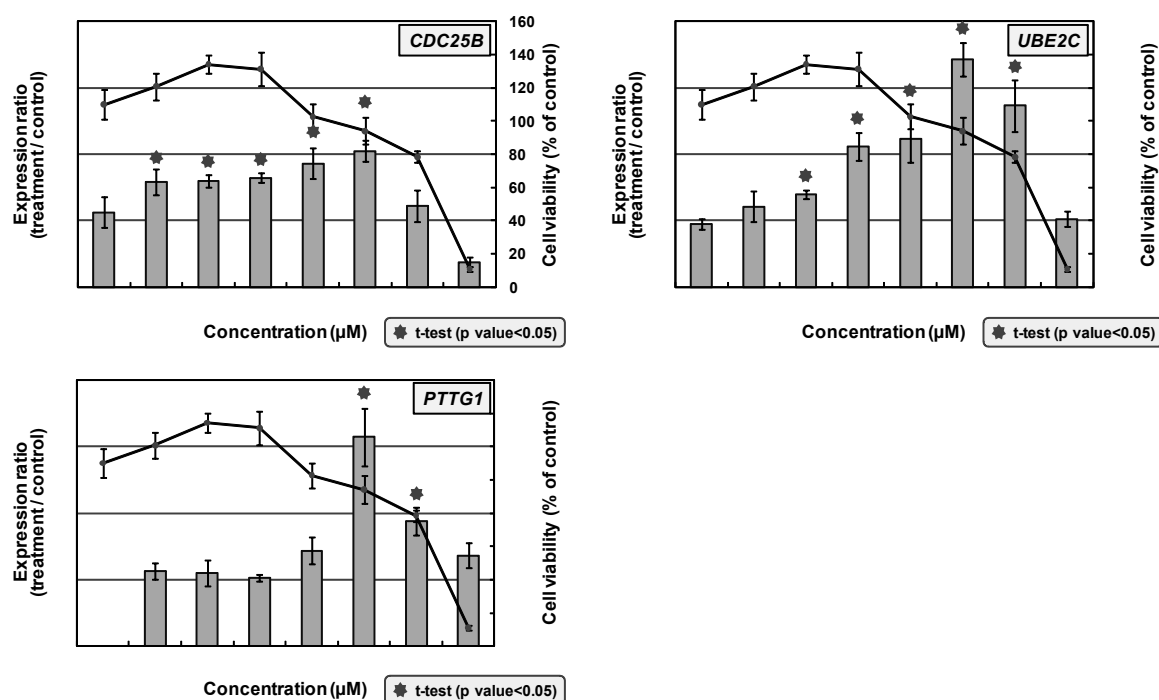


Fig. 5-4 Relative expression levels of CDC25B, UBE2C, and PTTG1 determined by RT-qPCR. Data represent the mean $\pm$ SD of triplicates. The line plots in each graph are identical, showing the cell viability.

DISCUSSION

Arsenic is an environmental pollutant and known human carcinogen. Although chronic arsenic exposure is strongly correlated with the occurrence of various diseases including cancers, the molecular mechanism underlying arsenic-induced cancers, particularly, in environmentally relevant exposure is not fully understood. Genomic instability has been claimed as a unique property that enables the acquisition of the cancerous hallmarks (25). A recent review by Bhattacharjee et al. (7) highlighted the major mechanisms that may be involved in arsenic-induced genomic instability, and concluded that oxidative stress produced by arsenic and its metabolites likely cause the genomic instability either at chromosomal level or nuclear level, through several mechanisms including DNA damage, inefficient DNA repair, telomere dysfunction, mitototic arrest, imperfect apoptosis (7). Those impacts by arsenic exposure were also reported in transcriptome-based studies. Bourdonnay et al. (9) and Perez et al. (10) observed the perturbation of genes particularly related to cell differentiation in human primary macrophages and human epidermal keratinocytes, respectively. Sasaki et al. (11) reported that the renal toxicity of arsenic was attributed to ROS production. Furthermore, Yu et al. (12) demonstrated arsenic induced apoptosis and cell cycle arrest in mouse embryonal fibroblasts (MEFs). So far, those carcinogenic actions of arsenic were, however, investigated in the limited concentration ranges (usually at lethal concentrations; hundreds nM or higher). Since the exposure of humans to arsenic is normally at a much lower level in daily life, the impacts of arsenic on the HepG2 cells was evaluated at the environmentally relevant concentrations (1nM-40 μ M) based on the by the DNA microarray-based transcriptome analysis.

Gene expression response of HepG2 exposed to inorganic arsenic at 6 μ M or higher well demonstrated that the molecular mechanisms of arsenic toxicity was similar to those of common heavy metals at cytotoxic concentrations, which has been characterized by increases in oxidative damage (i.e., HMOX1), glutathione (i.e., GPX3, GSR, GSTM3, GSTO1, GSS), heat shock proteins (i.e., HSPA1A, HSPA1B, HSPA4L, HSPB1, HSPH1), and metallothionein (i.e., MT1H, MT1X, MT2A) (26) (**Table 5-2**). Heat-shock proteins are known to be expressed in response to various stresses such as heat, low pH, cytotoxic chemicals and oxidative stress (27), while metallothionein is induced by the presence of heavy metals and stresses (28). Moreover, the induction of specific apoptotic genes (i.e., FAS, CASP3, CASP7, and CASP8) inferred receptor-mediated apoptosis via FADD adapter, probably triggered by Fas ligand which was produced by arsenic-induced oxygen radicals (29, 30). These results were supported by the result of ROS generation (**Fig. 5-2**).

Various genes categorized in cell cycle (CDC20, CDC25B, UBE2C, BIRC5, BUB1B, CKS1, and CKS2) were significantly induced at 0.07 - 6 μ M of arsenic (as low as the Japanese drinking water quality standard) (**Table 5-3**). This trend was commonly observed for model carcinogens. The induction of those cell cycle genes have been reported to trigger abnormal cell growth and enhance the progression of tumorigenesis (31,32,33,34,35,36). In fact, the induction of these cell cycle genes observed in 0.07-6 μ M exposures is in a good agreement with the increase in cell viability at these sub-lethal concentrations (**Fig. 5-1**). These results suggest that environmentally relevant exposures of inorganic arsenic could cause pro-carcinogenic impacts on the HepG2 cells. However, those genes were suppressed in 40 μ M exposure, leading to cell cycle arrest or apoptosis (37,38,39). This result was in concord with the significant induction of cell cycle arrest genes (CDKN1A and CDKN3) and apoptotic genes (CASP4, CASP7, CASP8, FAS, GADD45A, and GADD45B) (**Table 5-3**) and the significant reduction of cell viability at the high arsenic exposures (**Fig. 5-1**).

Furthermore, the suppression of the genes involved in DNA repair (RAD23B, CETN2) was observed from the lowest concentration (i.e., 0.07 μ M), suggesting the reduced DNA repair capacity. The oxidized DNA base lesions are known to be removed by two ways: base excision repair (BER) and nucleotide excision repair (NER) (40). NTHL1 and MUTYH encode the glycosylase detecting and removing the damaged base at the initial step of BER (40), XPC complex consisted of proteins encoded by RAD23B and CETN2 plays a critical role in NER (41). Present study suggested that arsenic exposure diminished the DNA repair capacity and enhanced cell proliferation at the same time, which was particularly significant in 0.5-6 μ M exposure. This clearly indicates the potential pro-carcinogenic actions of inorganic arsenic at environmentally relevant concentrations. It is speculated that the pro-carcinogenic actions of environmentally relevant

inorganic arsenic exposure may be further enhanced by the presence of other DNA damaging factors in the environment (e.g., oxidative stresses or UV radiation).

As discussed above, the present study suggested the environmentally relevant inorganic arsenic exposure (6 μM or even lower) could promote the spreading of genetic instability by accelerating cell proliferation. Based on this finding, we further evaluated the dose-response relationship between arsenic exposure and pro-carcinogenic effect by quantifying gene expression levels of the selected marker genes by the qPCR assay. The marker genes were selected based on the following three criteria: 1) induced at sub-lethal concentrations (i.e., 0.5 and 6 μM), 2) shared with the carcinogen promoter (TPA) and the carcinogen initiator (TCE), and 3) previously reported for the expression in tumors, or association with carcinogenesis and development of tumors. Among the genes satisfying the above criteria, CDC25B, UBE2C, and PTTG1 were selected as marker genes which likely represented pro-carcinogenic action of inorganic arsenic. CDC25B encodes one type of the Cell Division Cycle 258 (CDC25) phosphatase, which works as a checkpoint to arrest the cell cycle for DNA damages, and to reenter the cell into M phase after DNA repair (42). Thereby, its over-expression has been reported to cause tumorigenesis through proliferation of cells with genetic abnormality (43). UBE2C encodes the protein related to the ubiquitin-dependent proteolysis. Induction of UBE2C has been observed in cancers of ovary, hepatocyte, and prostate. It has been reported that the over-expression of this gene contributes to tumorigenesis by proliferating abnormal chromosom (44). PTTG1 regulates the transition from middle to late term of M phase, playing a key role in cellular differentiation and proliferation (45). The expression of PTTG1 has been observed in various tumors such as breast cancer and colon cancer (46, 47, 48).

The qPCR analysis demonstrated that the selected three genes were induced in a concentration –dependent manner (**Fig. 5-4**). Among those genes, UBE2C exhibited a clear concentration-dependent increase (0.5 - 6 μM) in its expression level (> 3-fold), suggesting the most promising genetic marker of inorganic arsenic exposure. CDC25B was significantly induced even at the lowest concentration (i.e., 0.07 μM), while PTTG1 was induced at sub-lethal concentrations (6 and 10 μM).

SUMMARY

In conclusion, the potential modes of toxic actions of inorganic arsenic were evaluated using DNA-microarray-based transcriptome analysis with human hepatocarcinoma HepG2 cells exposed to a wide concentration range of inorganic arsenic (5 nM to 40 μM). The transcriptome analysis revealed that the modes of toxic actions were clearly dependent upon the exposure concentration. The pro-carcinogenic actions (e.g., alternation of gene expression related to cell proliferation, cell cycle regulation, and signal transduction) of inorganic arsenic became more evident at environmentally relevant concentrations (below 6 μM). On the other hand, defense and stress-related actions (e.g., alternation of gene expression related to antioxidant components and metal binding and stress proteins) that commonly observed for heavy metals were observed at high exposure concentrations (10 μM). Furthermore, the genes related to cell cycle regulation (CDC25B,

UBE2C, and PTTG1) were selected as genetic markers for inorganic arsenic exposure, and it was confirmed that these gene were induced in a concentration-dependent manner. To our best knowledge, this study is the first to evaluate the inorganic arsenic toxicity at transcriptomic level in a wide range of exposure concentrations (5 nM to 40 μ M). The toxicogenomic data obtained in the present study will be useful for improvement of our knowledge of inorganic arsenic toxicity and the development of better strategies for human health risk assessment.

REFERENCES

- (1) Mandal, B. K.; Suzuki, K. T., Arsenic round the world: a review. *Talanta* 2002, 58 (1), 201-235.
- (2) Bissen, M.; Frimmel, F. H., Arsenic - a review. - Part 1: Occurrence, toxicity, speciation, mobility. *Acta Hydroch Hydrob* 2003, 31 (1), 9-18.
- (3) Some drinking-water disinfectants and contaminants, including arsenic; IARC monographs on the evaluation of carcinogenic risk of chemicals to humans vol. 84; International Agency for Research on Cancer (IARC): Lyon, France, 2004: <http://monographs.iarc.fr/ENG/Monographs/vol84/mono84-1.pdf>
- (4) Guha Mazumder, D.; Dasgupta, U. B., Chronic arsenic toxicity: studies in West Bengal, India. *Kaohsiung J Med Sci* 2011, 27 (9), 360-70.
- (5) Schuhmacher-Wolz, U.; Dieter, H. H.; Klein, D.; Schneider, K., Oral exposure to inorganic arsenic: evaluation of its carcinogenic and non-carcinogenic effects. *Crit Rev Toxicol* 2009, 39 (4), 271-298.
- (6) Salnikow, K.; Zhitkovich, A., Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: nickel, arsenic, and chromium. *Chem Res Toxicol* 2008, 21 (1), 28-44.
- (7) Bhattacharjee, P.; Banerjee, M.; Giri, A. K., Role of genomic instability in arsenic-induced carcinogenicity. A review. *Env. Int.* 2013, 53, 29-40
- (8) Ghosh, P.; Banerjee, M.; Giri, A. K.; Ray, K., Toxicogenomics of arsenic: Classical ideas and recent advances. *Mutat Res-Rev Mutat* 2008, 659 (3), 293-301.
- (9) Bourdonnay, E.; Morzadec, C.; Sparfel, L.; Galibert, M. D.; Jouneau, S.; Martin-Chouly, C.; Fardel, O.; Vernhet, L., Global effects of inorganic arsenic on gene expression profile in human macrophages. *Molecular Immunology* 2009, 46 (4), 649-656.
- (10) Perez, D. S.; Handa, R. J.; Yang, R. S. H.; Campain, J. A., Gene expression changes associated with altered growth and differentiation in benzo[a]pyrene or arsenic exposed normal human epidermal keratinocytes. *J Appl Toxicol* 2008, 28 (4), 491-508.
- (11) Sasaki, A.; Oshima, Y.; Fujimura, A., An approach to elucidate potential mechanism of renal toxicity of arsenic trioxide. *Exp Hematol* 2007, 35 (2), 252-62.
- (12) Yu, X.; Robinson, J. F.; Gribble, E.; Hong, S. W.; Sidhu, J. S.; Faustman, E. M., Gene expression profiling analysis reveals arseni-induced cell cycle arrest and apoptosis in p53-deficient cells through differential gene pathways. *Toxicol Appl Oharmacol* 2008, 233, 389-403
- (13) Shi, H.; Shi, X.; Liu, K. J., Oxidative mechanism of arsenic toxicity and carcinogenesis. *Mol Cell Biochem* 2004, 255 (1-2), 67-78.
- (14) Ruiz-Ramos, R.; Lopez-Carrillo, L.; Rios-Perez, A. D.; De Vizcaya-Ruiz, A.; Cebrian, M. E., Sodium arsenite induces ROS generation, DNA oxidative damage, HO-1 and c-Myc proteins, NF-kappaB activation and cell proliferation in human breast cancer MCF-7 cells. *Mutat Res* 2009, 674 (1-2), 109-115.
- (15) Kawata, K.; Osawa, M.; Okabe, S., In Vitro Toxicity of Silver Nanoparticles at Noncytotoxic Doses to HepG2 Human Hepatoma Cells. *Environ Sci Technol* 2009, 43 (15), 6046-6051.
- (16) Kawata, K.; Yokoo, H.; Shimazaki, R.; Okabe, S., Classification of heavy-metal toxicity by human DNA microarray analysis. *Environ Sci & Technol* 2007, 41 (10), 3769-3774.
- (17) Kawata, K.; Shimazaki, R.; Okabe, S., Comparison of gene expression profiles in HepG2 cells exposed to arsenic, cadmium, nickel, and three model carcinogens fro investigating the mechanisms of metal carcinogenesis. *Environ*

- Mol Mutagen 2009, 50 (1), 46-59.
- (18) Hara-Yamamura, H.; Nakashima, K.; Hoque, A.; Miyoshi, T.; Kimura, K.; Watanabe, Y.; Okabe, S. Evaluation of Whole Wastewater Effluent Impacts on HepG2 using DNA microarray-based Transcriptome Analysis, *Env. Sci. Tech.* 2013, 47(10), 5425-5432.
- (19) Mourot, M.; Dufort, I.; Gravel, C.; Algriany, O.; Dielealan, S.; Sirard, M. A., The influence of follicle size, FSH-enriched maturation medium, and early cleavage on bovine oocyte maternal mRNA levels. *Mol Reprod Dev* 2006, 73 (11), 1367-1379.
- (20) Doherty, S. C.; McKeown, S. R.; Lopez J. A.; Walsh, I. K.; McKelvey-Martin, V. J., Gene expression in normal urothelium depends on location within the bladder: a possible link to bladder carcinogenesis. *Eur Urol* 2006, 50 (2), 290-301.
- (21) Ieta, K.; Ojima, E.; Tanaka, F.; Nakamura, Y.; Haraguchi, N.; Mimori, K.; Inoue, H.; Kuwano, H.; Mori, M., Identification of overexpressed genes in hepatocellular carcinoma, with special reference to ubiquitin-conjugating enzyme E2C gene expression. *Int J Cancer* 2007, 121 (1), 33-38.
- (22) Xiong, Y.; Collins, Q. F.; An, J.; Lupo, E.; Liu, H. Y.; Liu, D. L.; Robidoux, J.; Liu, Z. Q.; Cao, W. H., p38 mitogen-activated protein kinase plays an inhibitory role in hepatic lipogenesis. *J Biol Chem* 2007, 282 (7), 4975-4982.
- (23) Chowdhury, R.; Chowdhury, S.; Roychoudhury, P.; Mandal, C.; Chaudhuri, K., Arsenic induced apoptosis in malignant melanoma cells is enhanced by menadione through ROS generation, p38 signaling and p53 activation. *Apoptosis* 2009, 14 (1), 108-123.
- (24) Ryu, B. M.; Qian, Z. J.; Kim, S. K., Purification of a peptide from seahorse, that inhibits arthritis-related cytokines through MAPK/NF-kappa B activation, and induces human osteoblastic and chondrocytic differentiation. *FEBS J* 2009, 276, 204-204.
- (25) Hanahan, D.; Winberg, R. A., The hallmarks of cancer. *Cell* 2000, 100, 57-70.
- (26) Koedrith, P.; Kim, H.; Weon, J.; Seo, Y. R., Toxicogenomic approaches for understanding molecular mechanism of heavy metal mutagenicity and carcinogenicity. *Int. J. Hyg. Envir. Heal* 2013, in press.
- (27) Lindquist, S.; Craig, E. A., The Heat-Shock Proteins. *Annu Rev Genet* 1988, 22, 631-677.
- (28) Coyle, P.; Philcox, J. C.; Careya, L. C.; Rofe, A. M., Metallothionein: The multipurpose protein. *Cell Mol Life Sci* 2002, 59 (4), 627-647.
- (29) Ashkenazi, A.; Dixit, V. M., Death receptors: Signaling and modulation. *Science* 1998, 28, 1305-1308.
- (30) Hug, H.; Strand, S.; Grambihler, A.; Galle, J.; Hack, V.; Stremmel, W.; Krammer, P. H.; Galle, P. R., Reactive oxygen intermediates are involved in the induction of CD95 ligand mRNA expression by cytostatic drugs in hepatoma cells. *J. Biol. Chem* 1997, 272 (45), 28191-28193.
- (31) Yuan, B. B.; Xu, Y.; Woo, J. H.; Wang, Y. Y.; Bae, Y. K.; Yoon, D. S.; Wersto, R. P.; Tully, E.; Wilsbach, K.; Gabrielson, E., Increased expression of mitotic checkpoint genes in breast cancer cells with chromosomal instability. *Clin Cancer Res* 2006, 12 (2), 405-410.
- (32) Kiyokawa, H.; Ray, D., In vivo roles of CDC25 phosphatases: biological insight into the anti-cancer therapeutic targets. *Anticancer Agents Med Chem* 2008, 8(8), 832-6.
- (33) Ieta, K.; Ojima, E.; Tanaka, F.; Nakamura, Y.; Haraguchi, N.; Mimori, K.; Inoue, H.; Kuwano, H.; Mori, M., Identification of overexpressed genes in hepatocellular carcinoma, with special reference to ubiquitin-conjugating enzyme E2C gene expression. *Int J Cancer* 2007, 121 (1), 33-38.
- (34) Boidot, R.; Vegran, F.; Jacob, D.; Chevrier, S.; Gangneux, N.; Taboureau, J.; Oudin, C.; Rainville, V.; Mercier, L.; Lizard-Nacol, S., The expression of BIRC5 is correlated with loss of specific chromosomal regions in breast carcinomas. *Gene Chromosome Canc* 2008, 47 (4), 299-308.
- (35) Scintu, M.; Vitale, R.; Prencipe, M.; Gallo, A. P.; Bonghi, L.; Valori, V. M.; Maiello, E.; Rinaldi, M.; Signori, E.; Rabitti, C.; Carella, M.; Dallapiccola, B.; Altomare, V.; Fazio, V. M.; Parrella, P., Genomic instability and increased

- expression of BUB1B and MAD2L1 genes in ductal breast carcinoma. *Cancer Lett* 2007, 254 (2), 298-307.
- (36) Lan, Y. S.; Zhang, Y. Y.; Wang, J. H.; Lin, C. H.; Ittmann, M. M.; Wang, F., Aberrant expression of Cks1 and Cks2 contributes to prostate tumorigenesis by promoting proliferation and inhibiting programmed cell death. *Int J Cancer* 2008, 123 (3), 543-551.
- (37) Hester, K. D.; Verhelle, D.; Escoubet-Lozach, L.; Luna, R.; Rose, D. W.; Glass, C. K., Differential repression of c-myc and cdc2 gene expression by ERF and PE-1/METS. *Cell Cycle* 2007, 6 (13), 1594-1604.
- (38) Ma, H. T.; Tsang, Y. H.; Marxer, M.; Poon, R. Y. C., Cyclin A2-Cyclin-Dependent Kinase 2 Cooperates with the PLK1-SCF beta-TrCP1-EMI1-Anaphase-Promoting Complex/Cyclosome Axis To Promote Genome Reduplication in the Absence of Mitosis. *Mol Cell Biol* 2009, 29 (24), 6500-6514.
- (39) Chesnel, F.; Bazile, F.; Pascal, A.; Kubiak, J. Z., Cyclin B2/cyclin-dependent kinase1 dissociation precedes CDK1 Thr-161 dephosphorylation upon M-phase promoting factor inactivation in *Xenopus laevis* cell-free extract. *Int J Dev Biol* 2007, 51 (4), 297-305.
- (40) Cook, M. S.; Evans, M. D.; Dizdaroglu, M.; Lunec, J., Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB* 2003, 17, 1195-1214.
- (41) Bunick, C. G.; Miller, M. R.; Fuller, B. E.; Fanning, E.; Chazin, J., Biochemical and structural domain analysis of xeroderma pigmentosum complementation group C protein. *Biochemistry* 2006, 45, 14965-14979.
- (42) Boutros, R.; Lobjois, V.; Ducommun, B., CDC25 phosphatases in cancer cells: key players? Good targets? *Nat Rev Cancer* 2007, 7 (7), 495-507.
- (43) Shou, J. Z.; Hu, N.; Takikita, M.; Roth, M. J.; Johnson, L. L.; Giffen, C.; Wang, Q. H.; Wang, C. Y.; Wang, Y.; Su, H.; Kong, L. H.; Emmert-Buck, M. R.; Goldstein, A. M.; Hewitt, S. M.; Taylor, P. R., Overexpression of CDC25B and LAMC2 mRNA and protein in esophageal squamous cell carcinomas and premalignant lesions in subjects from a high-risk population in China. *Cancer Epidem Biomar* 2008, 17 (6), 1424-1435.
- (44) Fujita, T.; Ikeda, H.; Taira, N.; Hatoh, S.; Naito, M.; Doihara, H., Overexpression of UbcH10 alternates the cell cycle profile and accelerate the tumor proliferation in colon cancer. *Bmc Cancer* 2009, 9 (87), 1-10.
- (45) Vlotides, G.; Eigler, T.; Melmed, S., Pituitary tumor-transforming gene: Physiology and implications for tumorigenesis. *Endocr Rev* 2007, 28 (2), 165-186.
- (46) Heaney, A. P.; Singson, R.; McCabe, C. J.; Nelson, V.; Nakashima, M.; Melmed, S., Expression of pituitary-tumour transforming gene in colorectal tumours. *Lancet* 2000, 355 (9205), 716-719.
- (47) Puri, R.; Tousson, A.; Chen, L. L.; Kakar, S. S., Molecular cloning of pituitary tumor transforming gene 1 from ovarian tumors and its expression in tumors. *Cancer Lett* 2001, 163 (1), 131-139.
- (48) Solbach, C.; Roller, M.; Fellbaum, C.; Nicoletti, M.; Kaufmann, M., PTTG mRNA expression in primary breast cancer: a prognostic marker for lymph node invasion and tumor recurrence. *Breast* 2004, 13 (1), 80-81.

7

CONCLUSION

The reclaimed wastewater has been served as an alternative water source in some countries. Although growing water stress, cost and energy constraints have prompted a call for more widespread utilization of wastewater reclamation and reuse practices, uncertain impacts of wastewater residues including vast categories of micropollutants remains as a challenge to increase the public acceptance. Due to its comprehensive and rapid nature for detecting early response of human cells, transcriptome analysis such as DNA microarray and quantitative RT-PCR assay, was applied to evaluate the whole effluent impacts and establish marker genes for the wastewater residues which may affect physiological processes of human hepatocarcinoma cells (HepG2). Furthermore, to better understand the effluent impacts at the transcriptome level, gene expression profiles of inorganic arsenic, a known contaminant in the water source of Sapporo, was also studied.

First, the DNA microarray-based transcriptome analysis with human HepG2 cells is a powerful tool to rapidly and comprehensively evaluate impacts of whole wastewater effluents. The potential genetic markers were also proposed to quantitatively evaluate the treatability of the wastewater effluents, which can be used to search for the physicochemical properties of the biologically active fraction of effluents. However, since the harmful impacts and innocuous impacts could not be distinguished at present, continued efforts are indispensable to identify some key biological processes and establish their relationship with some important endpoints.

Second, AKR1B10, CYP1A1, GPX2 and GCLM were selected as effluent-responsive marker genes among the commonly altered genes identified in the DNA microarray analysis of two or more effluents, based on their dose-response relationship in exposure of concentrated MBR and AS effluents. RO concentration was suggested as an effective pretreatment step for DNA microarray of relatively “clean” effluents.

Third, based on response of the selected marker genes, hydrophobic organic content, which may be forming complex with metals, were suggested as responsible fraction for a part of gene expression response observed in HepG2 exposed to MBR

and AS effluents (particularly CYP1A1-inducible fraction), and possibly behave together with humic substances or their building blocks with fell in a size range from 300 to thousands of Da. Thus, activated carbon adsorption or reverse osmosis were suggested as key processes in reclamation system of the selected MBR and AS effluents.

Finally, comparison of gene expression profiles between model chemicals and wastewater effluents revealed the uniqueness of the gene expression profiles of the effluents were suggested in comparison to model toxicants (i.e., heavy metals, oxidative stress inducers, and carcinogens).

Since there is still a gap between the gene expression response and the manifestation of toxicities, the gene expression response should be considered as "potential" of toxicity and thereby, further accumulation of DNA microarray data with well-established toxicity data is indispensable for development of this technology. In addition, due to the change of raw wastewater quality, seasonal variations of effluent impacts are to be investigated based on the gene expression response.

SUPPLEMENTAL MATERIALS

S1: Water quality of tested effluents

Forty water quality parameters of the National Effluent Standards (1) were measured in effluents from AS-MBR, S-MBRs, and AS. Six liters of water samples were taken into appropriately prepared bottles, and sent to the accredited analytical institute (Hiyoshi Ecological Services Co., Shiga, Japan) in cold storage. Analysis was carried out by using standard methods (2). The result summary is shown in Table 1.

Table 1 Effluent quality of effluents from three MBRs and AS

a) Parameters related to the protection of human health

Parameters	AS-MBR	S-MBR _A	S-MBR _B	AS	Influent*	Thresholds**
pH	7.5	7.4	7.1	6.8	7.4	5.8-8.6
BOD	6	30	11	7	130	160 mg/L
suspended solids	< 0.5	<0.5	<0.5	2.4	62	200 mg/L
n-hexane extracts (mineral oil)	<0.5	<0.5	<0.5	<0.5	22	30 mg/L
n-hexane extracts (animal and vegetable fats)	<0.5	<0.5	<0.5	<0.5		5 mg/L
Phenols	<0.1	<0.1	<0.1	<0.1	<0.5	5 mg/L
Copper	<0.01	<0.01	<0.01	<0.01	0.03	3 mg/L
Zinc	0.02	0.03	0.03	0.03	0.10	2 mg/L
Dissolved iron	<0.10	<0.10	<0.10	<0.10	1.8	10 mg/L
Dissolved manganese	<0.10	0.15	<0.10	<0.10	0.13	10 mg/L
Chromium	<0.01	<0.01	<0.01	<0.01	<0.005	2 mg/L
Nitrogen	9.9	10.7	15.2	7.2	27	120 mg/L (daily average 60 mg/L)
Phosphorus	0.1	0.6	0.3	0.1	2.7	16 mg/L (daily average 8 mg/L)

* Effluent from primary sedimentation basin; Gov. of Sapporo, 2012 (3)

** MoEJ, 1971 (1)

b) Parameters related to the protection of human health

Parameters	AS-MBR	S-MBR _A	S-MBR _B	AS	Influent*	Thresholds**
Cadmium and its compounds	<0.005	<0.005	<0.005	<0.005	<0.005	0.1 mg/L
Cyanide compounds	<0.01	<0.01	<0.01	<0.01	<0.01	1 mg/L
Organic phosphorus compounds	N/D	N/D	N/D	N/D	<0.05	1 mg/L
Lead and its compounds	<0.05	<0.05	<0.05	<0.05	<0.01	0.1 mg/L
hexavalent chrome compounds	<0.01	<0.01	<0.01	<0.01	<0.05	0.5 mg/L
Arsenic and its compounds	<0.01	<0.01	<0.01	<0.01	0.013	0.1 mg/L
Total mercury	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	0.005 mg/L
Alkyl mercury compounds	N/D	N/D	N/D	N/D	N/A	Not detected
PCBs	<0.0005	<0.0005	<0.0005	<0.0005	<0.00005	0.003 mg/L
Trichloroethylene	<0.001	<0.001	<0.001	<0.001	<0.001	0.3 mg/L
Tetrachloroethylene	<0.001	<0.001	<0.001	<0.001	<0.001	0.1 mg/L
Dichloromethane	<0.02	<0.02	<0.02	<0.02	<0.001	0.2 mg/L
Carbon tetrachloride	<0.001	<0.001	<0.001	<0.001	<0.001	0.02 mg/L
1,2-dichloro ethane	<0.004	<0.004	<0.004	<0.004	<0.001	0.04 mg/L
1,1-dichloro ethylene	<0.02	<0.02	<0.02	<0.02	<0.001	1mg/L
sis-1,2-dichloro ethylene	<0.04	<0.04	<0.04	<0.04	<0.001	0.4 mg/L
1,1,1-trichloro ethane	<0.001	<0.001	<0.001	<0.001	<0.001	3 mg/L
1,1,2-trichloro ethane	<0.006	<0.006	<0.006	<0.006	<0.001	0.06 mg/L
1,3-dichloropropene	<0.002	<0.002	<0.002	<0.002	<0.001	0.02 mg/L
Thiuram	<0.006	<0.006	<0.006	<0.006	<0.006	0.06 mg/L
Simazine	<0.003	<0.003	<0.003	<0.003	<0.002	0.03 mg/L
Thiobencarb	<0.02	<0.02	<0.02	<0.02	<0.02	0.2 mg/L
Benzene	<0.01	<0.01	<0.01	<0.01	<0.001	0.1 mg/L
Selenium and its compounds	<0.01	<0.01	<0.01	<0.01	<0.0005	0.1 mg/L
Boron and its compounds	0.3	0.4	0.3	0.3	0.2	10 mg/L
Fruorine and its compounds	<0.2	0.2	<0.2	<0.2	<0.2	8 mg/L
Ammonia, ammonium compounds, nitrate and nitrite compounds	9.5	10.5	15.2	6.5	8.2	100 mg/L

* Effluent from primary sedimentation basin; Gov. of Sapporo, 2012 (3)

** MoEJ, 1971 (1)

S2: Hierarchical Clustering Analysis and Principal Component Analysis of Overall Gene Expression data

The hierarchical clustering analysis using the Euclidean distance metric and principal component analysis (PCA) were performed on all the data sets obtained in this study using the entire genes on the microarray. The result of hierarchical clustering analysis is presented as a tree dendrogram (Fig. 2). In this tree, gene expression patterns were roughly classified into two clusters: 1) S-MBR effluents and the raw wastewater, and 2) AS-MBR effluent, AS effluent and the real drinking waters. Replicates of samples were mostly found in the same clusters except for tap water (TAP) and bottled water (BOT). PCA supported the result of the hierarchical clustering analysis (Fig. 3), showing the gene expression patterns of replicated samples were located close to each other.

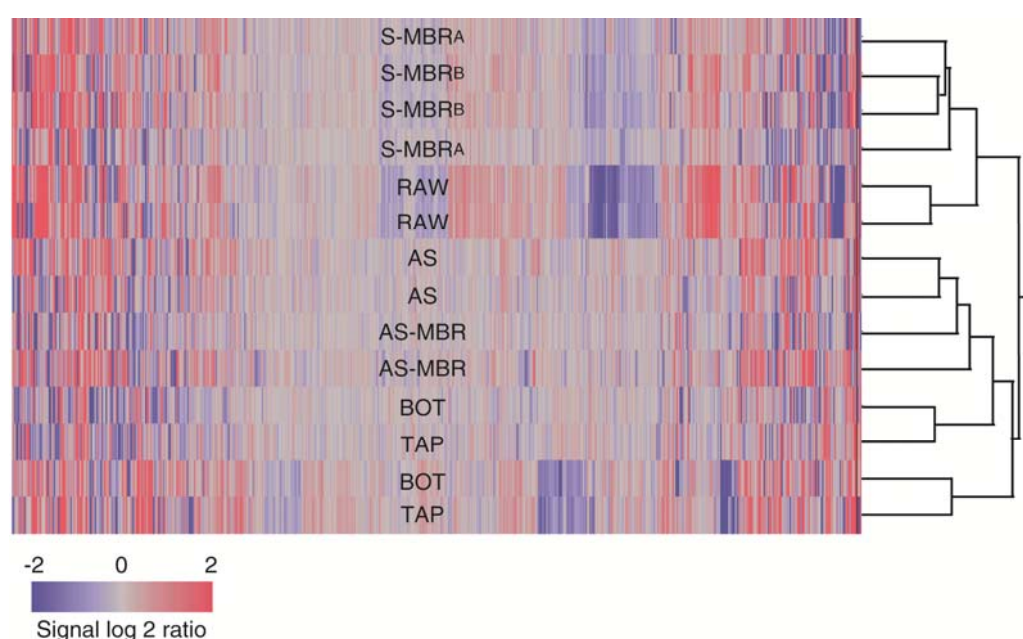


Fig. 2 Hierarchical clustering analysis of all the pairs of samples used in this study

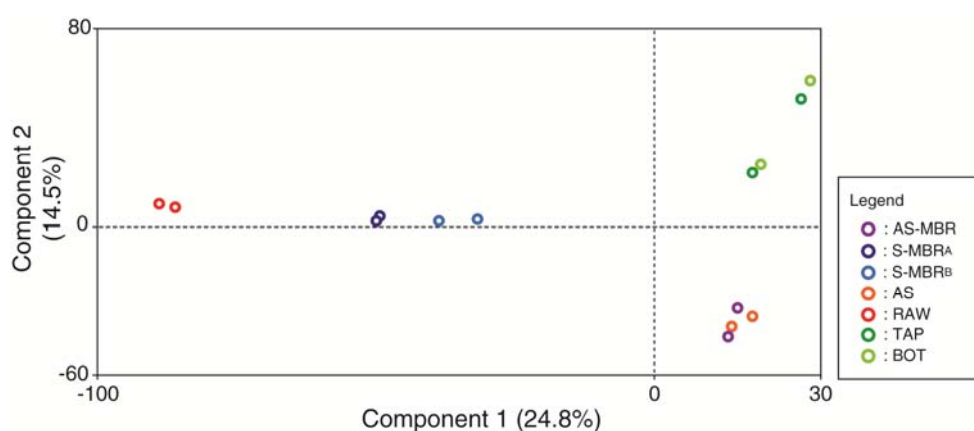


Fig. 3 Principle component analysis (PCA) based on the expression of selected genes (Variance of component 1 and 2 was 24.8% and 14.5%, respectively)

S3: Experimental Procedures of Conventional Bioassays

Bioluminescence inhibition test

Vibrio fischeri is naturally bioluminescent marine bacteria and their relative decrease in light output following to exposure to a tested sample has been used as an indicator of the toxic potency of a broad spectrum of compounds with different modes of action. Recently, several applications to the secondary sewage effluents and tertiary treated wastewater have been reported (4,5,6). The assay was conducted based on the standard procedure (ISO 11348-3) with modifications for a 96-well microplate (7). Briefly, reconstituted bacterial regent (Kyokuto Boeki Kaisha, Ltd., Tokyo, Japan) was incubated at laboratory ambient temperature before exposure. Prior to the exposure, 20 g/L NaCl was added in filtered water samples to adjusted saline content suitable for *V. fischeri*. Each replicate of the sample was tested at four different concentrations (i.e., 100%, 50%, 25% and 10%), in triplicate. Bioluminescence was quantified by microplate reader immediately prior to the exposure and then 5, 15 and 30 min after dosing.

Cytotoxicity tests

Cytotoxic effects of effluents on HepG2 cells were studied after 48 hour exposure based on the two different endpoints: 1) cell membrane integrity and 2) mitochondrial dehydrogenase activity (MTT assay). The direct cell counting with trypan blue dye exclusion was used to measure the number of the live cell with integrate membrane. After treatment, cells were trypsinized and collected from three individual dishes for every sample. The number of viable cells, which were not stained with trypan blue dye was counted by hepatocytometer (Onecell counter, Waken B Tech Co, LTD.). The mitochondrial dehydrogenase activity was determined by using MTT assay, which measured the amount of formazane resulted from reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT) (8). MTT (Sigma-Aldrich, Japan) dissolved in PBS was added onto the treated cells seeded in 96-well plates and incubated for 4 hours. Supernatant was removed after centrifugation for 5 min at 3000 rpm, and the amount of formazane pigment in DMSO (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) elutes was measured at 540 nm by plate reader (multilabel counter ARVO MX, PerkinElmer, US).

Statistical Analysis

Statistical analysis was performed by using Tukey's multivariate analysis. A P value of <0.05 was considered significant. Values were expressed as mean $\pm$ standard deviation of replicated experiments (n>3).

S4: Quantitative Real-Time Reverse-Transcriptase PCR (qPCR)

Selected primers are listed in Table 2. Primer sequences were derived from the literatures. Annealing temperatures were also determined based on the literatures with minor modifications. The relative expression of the selected genes for the same RNA sample as used in the DNA microarray, is shown in Fig. 2. The persistence of the alteration of the gene expressions was examined with the effluent samples taken on the different days, and the result is shown in Fig. 2.

Table 2 Sequences and annealing temperature of selected genes

Gene symbol	Sense primer	Antisense primer	T _{aneal}	Reference
<i>ADM</i>	CGGATGTCCAGCAGCTACCC	GTAGCCCTGGGGGCTGATCT	65	(9)
<i>AQP3</i>	CCTTTGGCTTTGCTGTCACTC	ACGGGGTTGTTGTAAGGGTCA	60	(10)
<i>DUSP1</i>	AGTACCCCACTCTACGATCAGG	TGATGGAGTCTATGAAGTCAATGG	55	(11)
<i>GCLC</i>	TTACCGAGGCTACGTGTCAGAC	TGTCGATGGTCAGGTCGATGTC	55	(12)
<i>STS</i>	AGGGTCTGGGTGTGTCTGTC	ACTGCAACGCCTACTTAAATG	60	(13)
<i>CYP39A1</i>	GTCTTCTGGACCCATTACCC	AGCTCAGGTCTAGGTGCTGC	64	(14)
<i>AKR1D1</i>	GGGGTGGTTGTCATTCTAA	GACTACCCATTGCACCGTCT	55	(15)

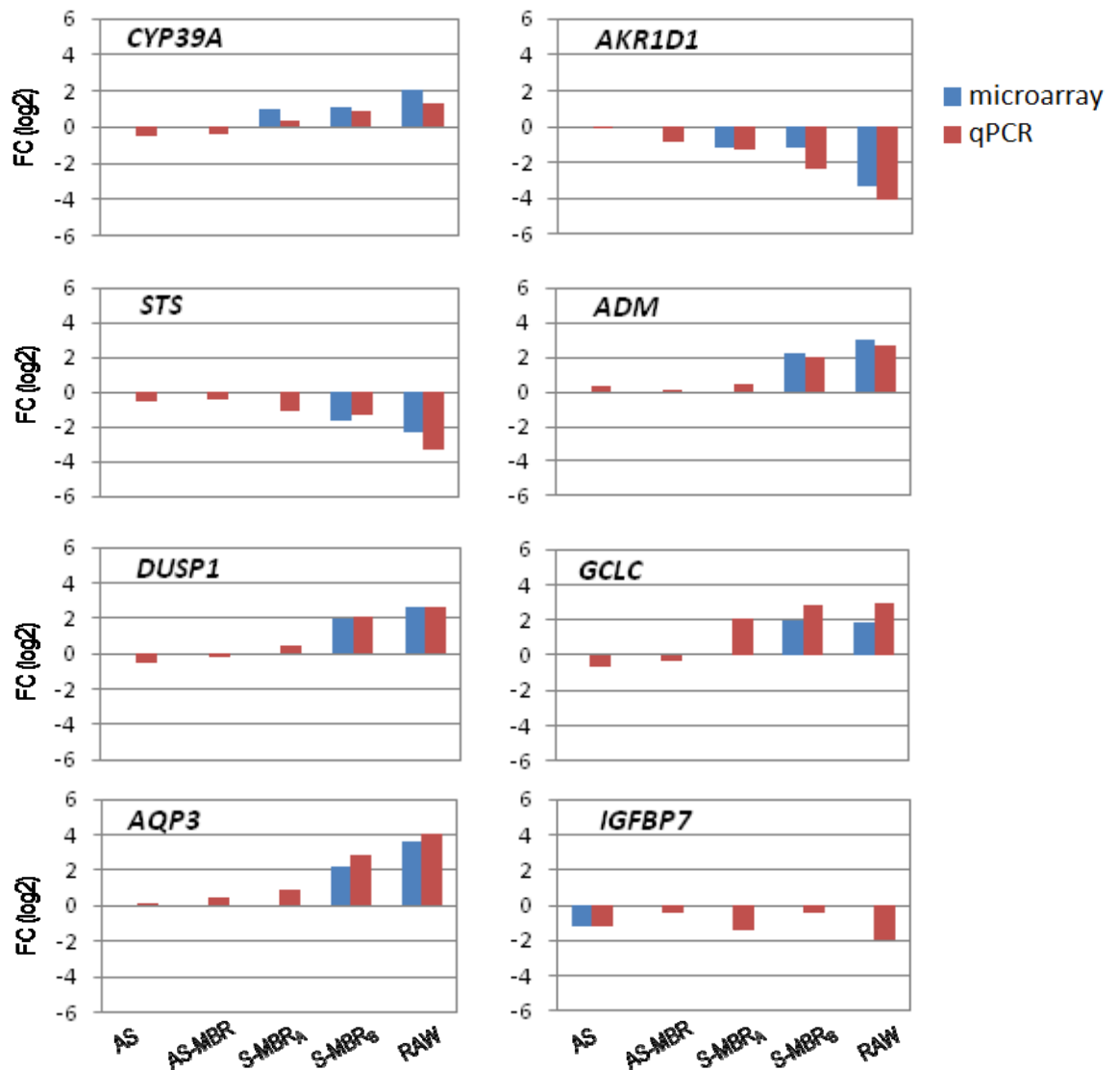


Fig. 4 Relative expression ratios of selected genes determined by DNA microarray and qPCR analysis

S5: Comprehensive List of Differentially Expressed Genes for Effluent Samples

a) AS-SMBR

Symbol	Gene name	FC (log2)	P-Value
<i>SQSTM1</i>	sequestosome 1	3.84	0.0194
<i>AKR1B10</i>	aldo-keto reductase family 1, member B10 (aldose reductase); aldo-keto reductase family 1, member B10-like	2.43	0.0016

b) S-MBR_A

Symbol	Gene name	FC (log2)	P-Value
<i>SQSTM1</i>	sequestosome 1	3.84	0.0194
<i>AKR1B10</i>	aldo-keto reductase family 1, member B10 (aldose reductase); aldo-keto reductase family 1, member B10-like	2.43	0.0016
<i>BHLHE40</i>	basic helix-loop-helix family, member e40	2.27	0.0050
<i>P2RY6</i>	pyrimidinergic receptor P2Y, G-protein coupled, 6	1.73	0.0037
<i>SERPINE1</i>	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	1.72	0.0021
<i>AKR1C2</i>	aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3- α hydroxysteroid dehydrogenase, type III)	1.68	0.0154
<i>EGR1</i>	early growth response 1	1.66	0.0013
<i>GCLM</i>	glutamate-cysteine ligase, modifier subunit	1.53	0.0084
<i>GPX2</i>	glutathione peroxidase 2 (gastrointestinal)	1.37	0.0005
<i>WDR13</i>	WD repeat domain 13	1.36	0.0285
<i>KLF4</i>	Kruppel-like factor 4 (gut)	1.30	0.0097
<i>DUSP13</i>	dual specificity phosphatase 13	1.29	0.0256
<i>SQSTM1</i>	sequestosome 1	1.29	0.0003
<i>PHLDA2</i>	pleckstrin homology-like domain, family A, member 2	1.22	0.0007
<i>ADCY7</i>	adenylate cyclase 7	1.06	0.0023
<i>CYP39A1</i>	cytochrome P450, family 39, subfamily A, polypeptide 1	1.02	0.0216
<i>TXNRD1</i>	thioredoxin reductase 1; hypothetical LOC100130902	1.00	0.0009
<i>DKK1</i>	dickkopf homolog 1 (<i>Xenopus laevis</i>)	-1.00	0.0240
<i>LEPR</i>	leptin receptor	-1.01	0.0442
<i>SLC16A4</i>	solute carrier family 16, member 4 (monocarboxylic acid transporter 5)	-1.02	0.0150
<i>PDZK1</i>	PDZ domain containing 1	-1.03	0.0103
<i>FABP1</i>	fatty acid binding protein 1, liver	-1.05	0.0197
<i>ICAM4</i>	intercellular adhesion molecule 4 (Landsteiner-Wiener blood group)	-1.06	0.0043
<i>FGB</i>	fibrinogen beta chain	-1.08	0.0330
<i>AKR1D1</i>	aldo-keto reductase family 1, member D1 (delta 4-3-ketosteroid-5-beta-reductase)	-1.16	0.0079
<i>IFIT1</i>	interferon-induced protein with tetratricopeptide repeats 1	-1.22	0.0222
<i>DPYD</i>	dihydropyrimidine dehydrogenase	-1.23	0.0004
<i>CHGB</i>	chromogranin B (secretogranin 1)	-1.25	0.0182
<i>GNMT</i>	glycine N-methyltransferase	-1.27	0.0343
<i>ELF5</i>	E74-like factor 5 (ets domain transcription factor)	-1.33	0.0188
<i>PLA2G1B</i>	phospholipase A2, group IB (pancreas)	-1.34	0.0067
<i>COL2A1</i>	collagen, type II, alpha 1	-1.35	0.0081
<i>SERPINA7</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7	-1.36	0.0054
<i>FGA</i>	fibrinogen alpha chain	-1.51	0.0011
<i>KDM5A</i>	lysine (K)-specific demethylase 5A	-1.52	0.0191
<i>SLC25A21</i>	solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21	-1.66	0.0342
<i>ENPP2</i>	ectonucleotide pyrophosphatase/phosphodiesterase 2	-1.85	0.0013
<i>UPK3A</i>	uroplakin 3A	-2.28	0.0097
<i>PRH1</i> /// <i>PRH2</i>	proline rich 4 (lacrimal)	-3.44	0.0003

b) S-MBR_B

Symbol	Gene name	FC (log2)	P-Value
<i>SQSTM1</i>	sequestosome 1	4.19	0.0105
<i>SERPINE1</i>	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	3.68	0.0006
<i>EGR1</i>	early growth response 1	3.55	0.0013
<i>MMP3</i>	matrix metalloproteinase 3 (stromelysin 1, progelatinase)	3.51	0.0171
<i>BHLHE40</i>	basic helix-loop-helix family, member e40	3.41	0.0023
<i>CCL20</i>	chemokine (C-C motif) ligand 20	3.02	0.0117
<i>EGR3</i>	early growth response 3	2.80	0.0063
<i>GEM</i>	GTP binding protein overexpressed in skeletal muscle	2.65	0.0068
<i>AKAP12</i>	A kinase (PRKA) anchor protein 12	2.63	0.0002
<i>C5AR1</i>	complement component 5a receptor 1	2.59	0.0038
<i>PHLDA2</i>	pleckstrin homology-like domain, family A, member 2	2.42	0.0006
<i>TNS1</i>	tensin 1	2.28	0.0181
<i>PYGB</i>	phosphorylase, glycogen; brain	2.27	0.0041
<i>AKR1B10</i>	aldo-keto reductase family 1, member B10 (aldose reductase); aldo-keto reductase family 1, member B10-like	2.27	0.0005
<i>CDA</i>	cytidine deaminase	2.27	0.0394
<i>ADM</i>	adrenomedullin	2.24	0.0001
<i>KLF4</i>	Kruppel-like factor 4 (gut)	2.24	0.0053
<i>WDR13</i>	WD repeat domain 13	2.23	0.0045
<i>RNASET2</i>	ribonuclease T2	2.20	0.0108
<i>AQP3</i>	aquaporin 3 (Gill blood group)	2.17	0.0074
<i>GPR109B</i>	niacin receptor 2; niacin receptor 1	2.16	0.0054
<i>KLF5</i>	Kruppel-like factor 5 (intestinal)	2.14	0.0057
<i>DUSP1</i>	dual specificity phosphatase 1	2.00	0.0050
<i>TNFRSF12A</i>	tumor necrosis factor receptor superfamily, member 12A	2.00	0.0049
<i>GCLC</i>	glutamate-cysteine ligase, catalytic subunit	1.98	0.0053
<i>IGFBP1</i>	insulin-like growth factor binding protein 1	1.96	0.0020
<i>RRAD</i>	Ras-related associated with diabetes	1.96	0.0325
<i>LIF</i>	leukemia inhibitory factor (cholinergic differentiation factor)	1.95	0.0258
<i>CD3D</i>	CD3d molecule, delta (CD3-TCR complex)	1.89	0.0161
<i>TIMP1</i>	TIMP metalloproteinase inhibitor 1	1.87	0.0068
<i>ATF3</i>	activating transcription factor 3	1.84	0.0144
<i>PRSS8</i>	protease, serine, 8	1.80	0.0223
<i>DUSP5</i>	dual specificity phosphatase 5	1.78	0.0290
<i>IER3</i>	immediate early response 3	1.76	0.0044
<i>AREG</i>	amphiregulin; amphiregulin B	1.73	0.0033
<i>IGF2 /// INS-IGF2</i>	insulin-like growth factor 2 (somatomedin A); insulin; INS-IGF2 readthrough transcript	1.67	0.0121
<i>HPCAL1</i>	hippocalcin-like 1	1.66	0.0054
<i>SQRDL</i>	sulfide quinone reductase-like (yeast)	1.65	0.0036
<i>ELF3</i>	E74-like factor 3 (ets domain transcription factor, epithelial-specific)	1.63	0.0066
<i>ENO2</i>	enolase 2 (gamma, neuronal)	1.63	0.0168
<i>KRT19</i>	junction plakoglobin	1.60	0.0120
<i>ZFP36</i>	zinc finger protein 36, C3H type, homolog (mouse)	1.56	0.0008
<i>SQSTM1</i>	sequestosome 1	1.54	0.0008
<i>AMPD3</i>	adenosine monophosphate deaminase (isoform E)	1.52	0.0216
<i>GCLM</i>	glutamate-cysteine ligase, modifier subunit	1.52	0.0160
<i>DKK4</i>	dickkopf homolog 4 (<i>Xenopus laevis</i>)	1.50	0.0194
<i>LOC55908</i>	hepatocellular carcinoma-associated gene TD26	1.49	0.0295
<i>LDLR</i>	low density lipoprotein receptor	1.48	0.0146

(continued)

Symbol	Gene name	FC (log2)	P-Value
<i>APOL1</i>	apolipoprotein L, 1	1.45	0.0132
<i>SLC16A5</i>	similar to MCT; solute carrier family 16, member 5 (monocarboxylic acid transporter 6)	1.44	0.0001
<i>EMP3</i>	epithelial membrane protein 3	1.41	0.0047
<i>KLF6</i>	Kruppel-like factor 6	1.41	0.0008
<i>AXL</i>	AXL receptor tyrosine kinase	1.40	0.0024
<i>GADD45B</i>	growth arrest and DNA-damage-inducible, beta	1.40	0.0108
<i>PFKFB3</i>	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	1.39	0.0133
<i>ADCY7</i>	adenylate cyclase 7	1.39	0.0283
<i>GCNT3</i>	glucosaminyl (N-acetyl) transferase 3, mucin type	1.39	0.0021
<i>PFKP</i>	phosphofructokinase, platelet	1.37	0.0110
<i>MAFF</i>	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	1.35	0.0034
<i>PLIN2</i>	adipose differentiation-related protein	1.34	0.0062
<i>DUSP13</i>	dual specificity phosphatase 13	1.33	0.0199
<i>JAG1</i>	jagged 1 (Alagille syndrome)	1.33	0.0017
<i>CDKN1A</i>	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.32	0.0210
<i>S100A6</i>	S100 calcium binding protein A6	1.32	0.0002
<i>RORA</i>	RAR-related orphan receptor A	1.31	0.0010
<i>EPHA2</i>	EPH receptor A2	1.30	0.0027
<i>IL4R</i>	interleukin 4 receptor	1.29	0.0066
<i>TXNRD1</i>	thioredoxin reductase 1; hypothetical LOC100130902	1.29	0.0003
<i>ARG2</i>	arginase, type II	1.29	0.0074
<i>CEACAM4</i>	carcinoembryonic antigen-related cell adhesion molecule 4	1.28	0.0012
<i>TNFSF9</i>	tumor necrosis factor (ligand) superfamily, member 9	1.26	0.0031
<i>STX3</i>	syntaxin 3	1.26	0.0086
<i>ASAP2</i>	ArfGAP with SH3 domain, ankyrin repeat and PH domain 2	1.25	0.0068
<i>GPRC5C</i>	G protein-coupled receptor, family C, group 5, member C	1.25	0.0038
<i>ASPH</i>	aspartate beta-hydroxylase	1.25	0.0130
<i>RAB3B</i>	RAB3B, member RAS oncogene family	1.22	0.0160
<i>FOSL1</i>	FOS-like antigen 1	1.22	0.0485
<i>IGFBP4</i>	insulin-like growth factor binding protein 4	1.19	0.0202
<i>RIT1</i>	Ras-like without CAAX 1	1.18	0.0114
<i>UPP1</i>	uridine phosphorylase 1	1.17	0.0045
<i>S100P</i>	S100 calcium binding protein P	1.17	0.0097
<i>SLC20A1</i>	solute carrier family 20 (phosphate transporter), member 1	1.16	0.0035
<i>TRIB1</i>	tribbles homolog 1 (Drosophila)	1.16	0.0050
<i>S100A14</i>	S100 calcium binding protein A14	1.16	0.0309
<i>MCL1</i>	myeloid cell leukemia sequence 1 (BCL2-related)	1.15	0.0101
<i>PPP1R15A</i>	protein phosphatase 1, regulatory (inhibitor) subunit 15A	1.14	0.0215
<i>HK2</i>	hexokinase 2 pseudogene; hexokinase 2	1.14	0.0090
<i>MKLN1</i>	muskelin 1, intracellular mediator containing kelch motifs	1.14	0.0122
<i>HSPA13</i>	heat shock protein 70kDa family, member 13	1.14	0.0065
<i>DTNA</i>	dystrobrevin, alpha	1.13	0.0240
<i>IER2</i>	immediate early response 2	1.13	0.0291
<i>SERPINB9</i>	serpin peptidase inhibitor, clade B (ovalbumin), member 9	1.12	0.0100
<i>ITGA10</i>	integrin, alpha 10	1.11	0.0036
<i>HIVEP2</i>	human immunodeficiency virus type I enhancer binding protein 2	1.11	0.0042
<i>HK1</i>	hexokinase 1	1.09	0.0224
<i>TRIB3</i>	tribbles homolog 3 (Drosophila)	1.08	0.0024
<i>IL18</i>	interleukin 18 (interferon-gamma-inducing factor)	1.07	0.0092
<i>RGS20</i>	regulator of G-protein signaling 20	1.07	0.0047
<i>HAVCR1</i>	hepatitis A virus cellular receptor 1	1.07	0.0419

(continued)

Symbol	Gene name	FC (log2)	P-Value
<i>CD58</i>	CD58 molecule	1.06	0.0049
<i>GBE1</i>	glucan (1,4- α -), branching enzyme 1	1.06	0.0128
<i>ETS2</i>	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	1.06	0.0266
<i>DNAJB9</i>	DnaJ (Hsp40) homolog, subfamily B, member 9	1.06	0.0089
<i>CYP39A1</i>	cytochrome P450, family 39, subfamily A, polypeptide 1	1.06	0.0429
<i>MASP1</i>	mannan-binding lectin serine peptidase 1 (C4/C2 activating component of Ra-reactive factor)	1.05	0.0143
<i>SEMA3C</i>	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	1.04	0.0366
<i>PNP</i>	nucleoside phosphorylase	1.04	0.0104
<i>FHL2</i>	four and a half LIM domains 2	1.04	0.0165
<i>ADAP2</i>	ArfGAP with dual PH domains 2	1.02	0.0166
<i>IL6R</i>	interleukin 6 receptor	1.02	0.0178
<i>NEDD9</i>	neural precursor cell expressed, developmentally down-regulated 9	1.01	0.0264
<i>HEY1</i>	hairy/enhancer-of-split related with YRPW motif 1	1.01	0.0032
<i>SFN</i>	stratifin	1.01	0.0133
<i>IKBKG</i>	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	1.00	0.0250
<i>PLAT</i>	plasminogen activator, tissue	1.00	0.0239
<i>NR1H4</i>	nuclear receptor subfamily 1, group H, member 4	-1.01	0.0087
<i>SLC1A2</i>	solute carrier family 1 (glial high affinity glutamate transporter), member 2	-1.01	0.0080
<i>SORD</i>	sorbitol dehydrogenase	-1.03	0.0026
<i>CPN1</i>	carboxypeptidase N, polypeptide 1	-1.03	0.0100
<i>GLT8D1</i>	glycosyltransferase 8 domain containing 1	-1.05	0.0067
<i>CYP4F3</i>	cytochrome P450, family 4, subfamily F, polypeptide 3	-1.05	0.0148
<i>SGK1</i>	serum/glucocorticoid regulated kinase 1	-1.05	0.0004
<i>SLC2A4RG</i>	SLC2A4 regulator	-1.06	0.0228
<i>SLCO2B1</i>	solute carrier organic anion transporter family, member 2B1	-1.06	0.0097
<i>B3GNT1</i>	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1; UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 2	-1.06	0.0100
<i>GPLD1</i>	glycosylphosphatidylinositol specific phospholipase D1	-1.07	0.0032
<i>PSMB9</i>	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	-1.07	0.0060
<i>MAP4K4</i>	mitogen-activated protein kinase kinase kinase kinase 4	-1.09	0.0089
<i>SERPINA6</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 6	-1.10	0.0051
<i>ACE2</i>	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	-1.11	0.0138
<i>MMP11</i>	matrix metalloproteinase 11 (stromelysin 3)	-1.14	0.0361
<i>IL1RAP</i>	interleukin 1 receptor accessory protein	-1.14	0.0071
<i>CDH2</i>	cadherin 2, type 1, N-cadherin (neuronal)	-1.17	0.0067
<i>ATP6V0E2</i>	ATPase, H ⁺ transporting V0 subunit e2	-1.17	0.0100
<i>ACSM3</i>	acyl-CoA synthetase medium-chain family member 3	-1.18	0.0074
<i>ZNF83</i>	zinc finger protein 83	-1.19	0.0322
<i>EHHADH</i>	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	-1.19	0.0144
<i>DGAT1</i>	diacylglycerol O-acyltransferase homolog 1 (mouse)	-1.20	0.0137
<i>CSAD</i>	cysteine sulfinic acid decarboxylase	-1.21	0.0054
<i>VDR</i>	vitamin D (1,25- dihydroxyvitamin D3) receptor	-1.21	0.0023
<i>IGSF1</i>	immunoglobulin superfamily, member 1	-1.21	0.0022
<i>MYLK</i>	myosin light chain kinase	-1.22	0.0039
<i>MTTP</i>	microsomal triglyceride transfer protein	-1.22	0.0066
<i>ADH6</i>	alcohol dehydrogenase 6 (class V)	-1.23	0.0116
<i>LGALS2</i>	lectin, galactoside-binding, soluble, 2	-1.24	0.0127

(continued)

Symbol	Gene name	FC (log2)	P-Value
<i>AKR1D1</i>	aldo-keto reductase family 1, member D1 (delta 4-3-ketosteroid-5-beta-reductase)	-1.24	0.0220
<i>FABP1</i>	fatty acid binding protein 1, liver	-1.25	0.0156
<i>BHMT</i>	betaine-homocysteine methyltransferase	-1.28	0.0186
<i>SULT2A1</i>	sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1	-1.28	0.0424
<i>SMPX</i>	small muscle protein, X-linked	-1.29	0.0476
<i>CCNG2</i>	cyclin G2	-1.30	0.0054
<i>ATP5C1</i>	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	-1.32	0.0173
<i>DDC</i>	dopa decarboxylase (aromatic L-amino acid decarboxylase)	-1.34	0.0040
<i>AGXT</i>	alanine-glyoxylate aminotransferase	-1.37	0.0132
<i>TNNC2</i>	troponin C type 2 (fast)	-1.38	0.0103
<i>ICAM4</i>	intercellular adhesion molecule 4 (Landsteiner-Wiener blood group)	-1.40	0.0018
<i>TNFSF10</i>	tumor necrosis factor (ligand) superfamily, member 10	-1.44	0.0054
<i>MT1X</i>	metallothionein 1X	-1.50	0.0005
<i>RGN</i>	regucalcin (senescence marker protein-30)	-1.51	0.0020
<i>RAB40B</i>	RAB40B, member RAS oncogene family	-1.55	0.0004
<i>PDE6D</i>	phosphodiesterase 6D, cGMP-specific, rod, delta	-1.60	0.0079
<i>WWOX</i>	WW domain containing oxidoreductase	-1.60	0.0177
<i>PDZK1</i>	PDZ domain containing 1	-1.62	0.0014
<i>STS</i>	steroid sulfatase (microsomal), isozyme S	-1.65	0.0177
<i>LOC100506517</i>	aldehyde dehydrogenase 6 family, member A1	-1.66	0.0080
<i>ELF5</i>	E74-like factor 5 (ets domain transcription factor)	-1.67	0.0115
<i>PLA2G1B</i>	phospholipase A2, group IB (pancreas)	-1.76	0.0084
<i>HPR</i>	haptoglobin-related protein; haptoglobin	-1.77	0.0057
<i>SERPINA7</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7	-1.77	0.0123
<i>SLC26A3</i>	solute carrier family 26, member 3	-1.80	0.0016
<i>FGB</i>	fibrinogen beta chain	-1.84	0.0152
<i>APOC3</i>	apolipoprotein C-III	-1.97	0.0023
<i>DPYD</i>	dihydropyrimidine dehydrogenase	-1.99	0.0013
<i>HP</i>	haptoglobin-related protein; haptoglobin	-2.01	0.0155
<i>COL2A1</i>	collagen, type II, alpha 1	-2.04	0.0033
<i>ENPP2</i>	ectonucleotide pyrophosphatase/phosphodiesterase 2	-2.12	0.0005
<i>FGA</i>	fibrinogen alpha chain	-2.49	0.0011
<i>KNG1</i>	kininogen 1	-2.97	0.0103
<i>UPK3A</i>	uroplakin 3A	-2.99	0.0099
<i>IFIT1</i>	interferon-induced protein with tetratricopeptide repeats 1	-3.12	0.0129
<i>GNMT</i>	glycine N-methyltransferase	-3.23	0.0200

C) AS

Symbol	Gene name	FC (log2)	P-Value
<i>GNAO1</i>	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O	2.36	0.0008
<i>AVPR1A</i>	arginine vasopressin receptor 1A	2.11	0.0004
<i>UGT1A10</i> /// <i>UGT1A7</i> /// <i>UGT1A8</i>	UDP glucuronosyltransferase 1 family, polypeptide A3; UDP glucuronosyltransferase 1 family, polypeptide A5; UDP glucuronosyltransferase 1 family, polypeptide A4; UDP glucuronosyltransferase 1 family, polypeptide A7; UDP glucuronosyltransferase 1 family, polypeptide A6; UDP glucuronosyltransferase 1 family, polypeptide A10; UDP glucuronosyltransferase 1 family, polypeptide A9; UDP glucuronosyltransferase 1 family, polypeptide A8; UDP glucuronosyltransferase 1 family, polypeptide A1	1.73	0.0025
<i>NPY2R</i>	neuropeptide Y receptor Y2	1.50	0.0212
<i>GPX2</i>	glutathione peroxidase 2 (gastrointestinal)	1.40	0.0350
<i>PPARA</i>	peroxisome proliferator-activated receptor alpha	1.14	0.0091
<i>PDGFD</i>	platelet derived growth factor D	1.12	0.0001
<i>HOXB7</i>	homeobox B7	1.12	0.0472
<i>OSGIN1</i>	oxidative stress induced growth inhibitor 1	1.10	0.0471
<i>IRAK4</i>	interleukin-1 receptor-associated kinase 4	-1.00	0.0125
<i>PLP1</i>	proteolipid protein 1	-1.03	0.0105
<i>ACADSB</i>	acyl-Coenzyme A dehydrogenase, short/branched chain	-1.10	0.0482
<i>IGFBP7</i>	insulin-like growth factor binding protein 7	-1.15	0.0358
<i>MT1X</i>	metallothionein 1X	-1.24	0.0412
<i>MBNL1</i>	muscleblind-like (Drosophila)	-1.25	0.0189
<i>CYR61</i>	cysteine-rich, angiogenic inducer, 61	-1.31	0.0099
<i>RAB3B</i>	RAB3B, member RAS oncogene family	-1.38	0.0340
<i>ANKRD1</i>	ankyrin repeat domain 1 (cardiac muscle)	-1.41	0.0264
<i>PRH1</i> /// <i>PRH2</i>	proline rich 4 (lacrimal)	-1.64	0.0151
<i>RRAS</i>	related RAS viral (r-ras) oncogene homolog	-2.32	0.0156

S6: Additional References

- (1) Ministry of the Environment, Japan. Ordinance of Prime Minister's Office Stipulating Effluent Standards, 1971
- (2) Ministry of the Environment, Japan. Verification Methods Stipulated by Minister of the Environment Based on Ministerial Ordinance Stipulating Effluent Standards, 1974
- (3) Department of Sewer System, Construction Bureau, City of Sapporo, Japan. Annual Maintenance Management Report of Sewage System in Sapporo, 2012
- (4) la Farre, M.; Garcia, M. J.; Tirapu, L.; Ginebreda, A.; Barcelo, D., Wastewater toxicity screening of non-ionic surfactants by Toxalert (R) and Microtox (R) bioluminescence inhibition assays. *Anal Chim Acta* 2001, 427 (2), 181-189.
- (5) Macova, M.; Escher, B. I.; Reungoat, J.; Carswell, S.; Chue, K. L.; Keller, J.; Mueller, J. F., Monitoring the biological activity of micropollutants during advanced wastewater treatment with ozonation and activated carbon filtration. *Water Research* 2010, 44 (2), 477-492.
- (6) Wei, D. B.; Wang, L. S.; Wei, J.; Hu, H. Y., Toxicity screening and evaluating in chlorination disinfection of wastewater reclamation processes. *Water Sci Technol* 2006, 53 (9), 239-246.
- (7) Escher, B. I.; Bramaz, N.; Mueller, J. F.; Quayle, P.; Rutishauser, S.; Vermeirssen, E. I. M., Toxic equivalent

- concentrations (TEQs) for baseline toxicity and specific modes of action as a tool to improve interpretation of ecotoxicity testing of environmental samples. *J Environ Monit* 2008, 10 (5), 612-621.
- (8) Carmichael, J.; Degraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B., Evaluation of a Tetrazolium-Based Semiautomated Colorimetric Assay - Assessment of Chemosensitivity Testing. *Cancer Res* 1987, 47 (4), 936-942.
 - (9) Linscheid, P.; Seboek, D.; Zulewski, H.; Keller, U.; Muller, B., Autocrine/paracrine role of inflammation-mediated calcitonin gene-related peptide and adrenomedullin expression in human adipose tissue. *Endocrinology* 2005, 146 (6), 2699-2708.
 - (10) Gresz, V.; Kwon, T. H.; Hurley, P. T.; Varga, G.; Zelles, T.; Nielsen, S.; Case, R. M.; Steward, M. C., Identification and localization of aquaporin water channels in human salivary glands. *Am J Physiol-Gastr L* 2001, 281 (1), G247-G254.
 - (11) Rauhala, H. E.; Porkka, K. P.; Tolonen, T. T.; Martikainen, P. M.; Tammela, T. L. J.; Visakorpi, T., Dual-specificity phosphatase 1 and serum/glucocorticoid-regulated kinase are downregulated in prostate cancer. *Int J Cancer* 2005, 117 (5), 738-745.
 - (12) Dasgupta, A.; Das, S.; Sarkar, P. K., Thyroid hormone promotes glutathione synthesis in astrocytes by up regulation of glutamate cysteine ligase through differential stimulation of its catalytic and modulator subunit mRNAs. *Free Radical Bio Med* 2007, 42 (5), 617-626.
 - (13) Suzuki, T.; Nakata, T.; Miki, Y.; Kaneko, C.; Moriya, T.; Ishida, T.; Akinaga, S.; Hirakawa, H.; Kimura, M.; Sasano, H., Estrogen sulfotransferase and steroid sulfatase in human breast carcinoma. *Cancer Res* 2003, 63 (11), 2762-2770.
 - (14) Steckelbroeck, S.; Watzka, M.; Lutjohann, D.; Makiola, P.; Nassen, A.; Hans, V. H. J.; Clusmann, H.; Reissinger, A.; Ludwig, M.; Siekmann, L.; Klingmuller, D., Characterization of the dehydroepiandrosterone (DHEA) metabolism via oxysterol 7 alpha-hydroxylase and 17-ketosteroid reductase activity in the human brain. *J Neurochem* 2002, 83 (3), 713-726.
 - (15) Drury, J. E.; Mindnich, R.; Penning, T. M., Characterization of Disease-related 5 beta-Reductase (AKR1D1) Mutations Reveals Their Potential to Cause Bile Acid Deficiency. *J Biol Chem* 2010, 285 (32), 24529-24537.

S7: List of Differentially Expressed Genes for Arsenic Ixposure

1 a) Up-regulated genes in 0.005 µM exposure

Symbol	Gene name	Fold cahnge
<i>SLC2A3</i>	solute carrier family 2 (facilitated glucose transporter), member 3	5.14
<i>GLRX</i>	glutaredoxin (thioltransferase)	3.98
<i>SLC6A8</i>	solute carrier family 6 (neurotransmitter transporter, creatine), member 8	3.18
<i>RPL31</i>	ribosomal protein L31	3.02
<i>SEC24D</i>	SEC24 related gene family, member D (S. cerevisiae)	2.88
<i>RARS</i>	arginyl-tRNA synthetase	2.41
<i>MAD2L1</i>	MAD2 mitotic arrest deficient-like 1 (yeast)	2.34
<i>PNRC1</i>	proline-rich nuclear receptor coactivator 1	2.34
<i>ASMTL</i>	acetylserotonin O-methyltransferase-like	2.33
<i>IGFBP3</i>	insulin-like growth factor binding protein 3	2.32
<i>MAT2A</i>	methionine adenosyltransferase II, alpha	2.25
<i>FHL2</i>	four and a half LIM domains 2	2.16
<i>HIBCH</i>	3-hydroxyisobutryl-Coenzyme A hydrolase	2.15
<i>UBL3</i>	ubiquitin-like 3	2.14
<i>TAGLN</i>	transgelin	2.13
<i>INHBC</i>	inhibin, beta C	2.10
<i>ARL4A</i>	ADP-ribosylation factor-like 4A	2.09
<i>CKAP4</i>	cytoskeleton-associated protein 4	2.07
<i>ACTL6A</i>	actin-like 6A	2.05
<i>SSR1</i>	signal sequence receptor, alpha (translocon-associated protein alpha)	2.04
<i>COTL1</i>	coactosin-like 1 (Dictyostelium)	2.03

1 b) Down-regulated genes in 0.005 µM exposure

Symbol	Gene name	Fold cahnge
<i>PGC</i>	progastricsin (pepsinogen C)	3.67
<i>SLC6A14</i>	solute carrier family 6 (amino acid transporter), member 14	3.64
<i>IL6ST</i>	interleukin 6 signal transducer (gp130, oncostatin M receptor)	3.57
<i>SLC17A2</i>	solute carrier family 17 (sodium phosphate), member 2	2.99
<i>GSTA1</i>	glutathione S-transferase A1	2.75
<i>HOXD1</i>	homeobox D1	2.63
<i>SCGN</i>	secretagogin, EF-hand calcium binding protein	2.57
<i>F13B</i>	coagulation factor XIII, B polypeptide	2.46
<i>CLDN1</i>	claudin 1	2.46
<i>RAB17</i>	RAB17, member RAS oncogene family	2.43
<i>UBD</i>	ubiquitin D	2.40
<i>UGT2B4</i>	UDP glucuronosyltransferase 2 family, polypeptide B4	2.39
<i>CNNM1</i>	cyclin M1	2.35
<i>MET</i>	met proto-oncogene (hepatocyte growth factor receptor)	2.32
<i>NKTR</i>	natural killer-tumor recognition sequence	2.31
<i>EPHA2</i>	EPH receptor A2	2.31
<i>TIMP3</i>	TIMP metallopeptidase inhibitor 3 (Sorsby fundus dystrophy, pseudoinflammatory)	2.26
<i>MAT1A</i>	methionine adenosyltransferase I, alpha	2.24
<i>NR1H4</i>	nuclear receptor subfamily 1, group H, member 4	2.23
<i>SLC44A4</i>	solute carrier family 44, member 4	2.17
<i>NR0B2</i>	nuclear receptor subfamily 0, group B, member 2	2.13
<i>ICAM1</i>	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	2.11
<i>FXYD2 /// MB</i>	FXYD domain containing ion transport regulator 2 /// myoglobin	2.09
<i>C4BPA</i>	complement component 4 binding protein, alpha	2.09
<i>OSMR</i>	oncostatin M receptor	2.09
<i>GPX2</i>	glutathione peroxidase 2 (gastrointestinal)	2.08
<i>MBL2</i>	mannose-binding lectin (protein C) 2, soluble (opsonic defect)	2.07
<i>HIST2H2AA3 /// HIST2H2AA4</i>	histone cluster 2, H2aa3 /// histone cluster 2, H2aa4	2.05
<i>PRKAB1</i>	protein kinase, AMP-activated, beta 1 non-catalytic subunit	2.05
<i>EGR1</i>	early growth response 1	2.05
<i>PRKAB2</i>	protein kinase, AMP-activated, beta 2 non-catalytic subunit	2.04
<i>TNFRSF10D</i>	tumor necrosis factor receptor superfamily, member 10d, decoy with truncated death domain	2.04
<i>STC2</i>	stanniocalcin 2	2.03
<i>PPP4C</i>	protein phosphatase 4 (formerly X), catalytic subunit	2.02

2 a) Up-regulated genes in 0.07 µM exposure

Symbol	Gene name	Fold cahnge
<i>SLC2A3</i>	solute carrier family 2 (facilitated glucose transporter), member 3	10.03
<i>MAP2K2</i>	mitogen-activated protein kinase kinase 2	5.68
<i>FLNA</i>	filamin A, alpha (actin binding protein 280)	5.07
<i>TGFB1</i>	transforming growth factor, beta 1 (Camurati-Engelmann disease)	4.64
<i>CYR61</i>	cysteine-rich, angiogenic inducer, 61	4.50
<i>PTOV1</i>	prostate tumor overexpressed gene 1	4.20
<i>HMGA1</i>	high mobility group AT-hook 1	4.04
<i>CDC42EP1</i>	CDC42 effector protein (Rho GTPase binding) 1	3.71
<i>LRP5</i>	low density lipoprotein receptor-related protein 5	3.58
<i>TSC2</i>	tuberous sclerosis 2	3.52
<i>SHB</i>	Src homology 2 domain containing adaptor protein B	3.43
<i>TMEM8</i>	transmembrane protein 8 (five membrane-spanning domains)	3.41
<i>ZYX</i>	zyxin	3.34
<i>MGAT4B</i>	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme B	3.26
<i>HAMP</i>	hepcidin antimicrobial peptide	3.20
<i>SOLH</i>	small optic lobes homolog (Drosophila)	3.14
<i>EHMT2</i>	euchromatic histone-lysine N-methyltransferase 2	3.12
<i>CRLF1</i>	cytokine receptor-like factor 1	3.11
<i>PCQAP</i>	PC2 (positive cofactor 2, multiprotein complex) glutamine/Q-rich-associated protein	3.10
	CDNA clone IMAGE:3030163	3.09
<i>EHD1</i>	EH-domain containing 1	3.08
<i>CALM3</i>	calmodulin 3 (phosphorylase kinase, delta)	3.07
<i>CKB</i>	creatine kinase, brain	2.94
<i>HGS</i>	hepatocyte growth factor-regulated tyrosine kinase substrate	2.93
<i>M6PRBP1</i>	mannose-6-phosphate receptor binding protein 1	2.93
<i>COTL1</i>	coactosin-like 1 (Dictyostelium)	2.88
<i>COL9A3</i>	collagen, type IX, alpha 3	2.81
<i>CNOT3</i>	CCR4-NOT transcription complex, subunit 3	2.81
<i>NDRG1</i>	N-myc downstream regulated gene 1	2.80
<i>VAR5</i>	valyl-tRNA synthetase	2.80
<i>SLC6A8</i>	solute carrier family 6 (neurotransmitter transporter, creatine), member 8	2.80
<i>VAT1</i>	vesicle amine transport protein 1 homolog (T. californica)	2.80
<i>FHL2</i>	four and a half LIM domains 2	2.76
<i>TPM4</i>	tropomyosin 4	2.75
<i>LSR</i>	lipolysis stimulated lipoprotein receptor	2.69
<i>TAGLN</i>	transgelin	2.67
<i>CDC25B</i>	cell division cycle 25 homolog B (S. cerevisiae)	2.62
<i>PGF</i>	placental growth factor, vascular endothelial growth factor-related protein	2.60
<i>SPTAN1</i>	spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	2.59
<i>BHLHB2</i>	basic helix-loop-helix domain containing, class B, 2	2.59
<i>SPOP</i>	speckle-type POZ protein	2.58
<i>DCTN1</i>	dynactin 1 (p150, glued homolog, Drosophila)	2.58
<i>CKAP4</i>	cytoskeleton-associated protein 4	2.57
<i>SERPINH1</i>	serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	2.51
<i>SPON2</i>	spondin 2, extracellular matrix protein	2.49
<i>STRN4</i>	striatin, calmodulin binding protein 4	2.48

Symbol	Gene name	Fold cahnge
<i>H1FX</i>	H1 histone family, member X	2.47
<i>TNFRSF21</i>	tumor necrosis factor receptor superfamily, member 21	2.47
<i>FASN</i>	fatty acid synthase	2.45
<i>DUS1L</i>	dihydrouridine synthase 1-like (S. cerevisiae)	2.44
<i>GNB2</i>	guanine nucleotide binding protein (G protein), beta polypeptide 2	2.44
<i>POLRMT</i>	polymerase (RNA) mitochondrial (DNA directed)	2.44
<i>BRD4</i>	bromodomain containing 4	2.44
<i>LMNA</i>	lamin A/C	2.41
<i>CHST3</i>	carbohydrate (chondroitin 6) sulfotransferase 3	2.40
<i>PPM1G</i>	protein phosphatase 1G (formerly 2C), magnesium-dependent, gamma isoform	2.39
<i>SCARB1</i>	scavenger receptor class B, member 1	2.39
<i>CBX4</i>	chromobox homolog 4 (Pc class homolog, Drosophila)	2.39
<i>PLOD3</i>	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3	2.36
<i>HDGF</i>	hepatoma-derived growth factor (high-mobility group protein 1-like)	2.36
<i>TMC6</i>	transmembrane channel-like 6	2.34
<i>DVL3</i>	dishevelled, dsh homolog 3 (Drosophila)	2.32
<i>PKM2</i>	pyruvate kinase, muscle	2.32
<i>DULLARD</i>	dullard homolog (Xenopus laevis) /// dullard homolog (Xenopus laevis)	2.32
<i>ST6GALNAC4</i>	ST6 (alpha-N-acetyl-neuraminy-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 4	2.31
<i>GBF1</i>	golgi-specific brefeldin A resistance factor 1	2.31
<i>TRIM28</i>	tripartite motif-containing 28	2.31
<i>GAA</i>	glucosidase, alpha; acid (Pompe disease, glycogen storage disease type II)	2.29
<i>UBXD1</i>	UBX domain containing 1	2.29
<i>RAB5C</i>	RAB5C, member RAS oncogene family	2.29
<i>HRAS</i>	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	2.29
<i>PNKP</i>	polynucleotide kinase 3'-phosphatase	2.28
<i>SF1</i>	splicing factor 1	2.26
<i>ASMTL</i>	acetylserotonin O-methyltransferase-like	2.26
<i>RPS6KA1</i>	ribosomal protein S6 kinase, 90kDa, polypeptide 1	2.25
<i>TLE3</i>	transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)	2.25
<i>PLK1</i>	polo-like kinase 1 (Drosophila)	2.22
<i>BAT3</i>	HLA-B associated transcript 3	2.21
<i>PRKCSH</i>	protein kinase C substrate 80K-H	2.19
<i>SERPINE1</i>	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	2.18
<i>PTBP1</i>	polypyrimidine tract binding protein 1	2.17
<i>HSPB1</i> /// <i>MEIS3</i>	heat shock 27kDa protein 1 /// Meis1, myeloid ecotropic viral integration site 1 homolog 3 (mouse)	2.17
<i>ALDH4A1</i>	aldehyde dehydrogenase 4 family, member A1	2.16
<i>RHOG</i>	ras homolog gene family, member G (rho G)	2.15
<i>COL9A2</i>	collagen, type IX, alpha 2	2.15
<i>KDEL1</i>	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 1	2.11
<i>MFHAS1</i>	malignant fibrous histiocytoma amplified sequence 1	2.10
<i>CTBP1</i>	C-terminal binding protein 1	2.10
<i>TNIP1</i>	TNFAIP3 interacting protein 1	2.10
<i>C8orf55</i>	chromosome 8 open reading frame 55	2.09
<i>AKT1</i>	v-akt murine thymoma viral oncogene homolog 1	2.09
<i>FLII</i>	flightless I homolog (Drosophila)	2.07

Symbol	Gene name	Fold cahnge
<i>UBL3</i>	ubiquitin-like 3	2.07
<i>CDC20</i>	cell division cycle 20 homolog (S. cerevisiae)	2.06
<i>RBP1</i>	retinol binding protein 1, cellular	2.05
<i>HSPA1A</i> /// <i>HSPA1B</i>	heat shock 70kDa protein 1A /// heat shock 70kDa protein 1B	2.04
<i>SLC16A3</i>	solute carrier family 16, member 3 (monocarboxylic acid transporter 4)	2.04
	CDNA FLJ34214 fis, clone FCBBF3021807	2.04
<i>GCS1</i>	glucosidase I	2.03
<i>KHSRP</i>	KH-type splicing regulatory protein (FUSE binding protein 2)	2.03
<i>GPX3</i>	glutathione peroxidase 3 (plasma)	2.03
<i>LYPLA3</i>	lysophospholipase 3 (lysosomal phospholipase A2)	2.02
<i>GOLGA2</i>	golgi autoantigen, golgin subfamily a, 2	2.02
<i>SF3B4</i>	splicing factor 3b, subunit 4, 49kDa	2.02
<i>CNN2</i>	calponin 2	2.01
<i>MAP3K11</i>	mitogen-activated protein kinase kinase kinase 11	2.01
<i>HPCAL1</i>	hippocalcin-like 1	2.01
<i>TUBB3</i>	tubulin, beta 3	2.01
<i>BCR</i>	breakpoint cluster region	2.01
<i>SH3BP4</i>	SH3-domain binding protein 4	2.00

2 b) Down-regulated genes in 0.07 µM exposure

Symbol	Gene name	Fold cahnge
<i>UGT2B4</i>	UDP glucuronosyltransferase 2 family, polypeptide B4	3.58
<i>SLC17A2</i>	solute carrier family 17 (sodium phosphate), member 2	3.44
<i>SCGN</i>	secretagogin, EF-hand calcium binding protein	3.38
<i>UBD</i>	ubiquitin D	3.21
<i>DPYD</i>	dihydropyrimidine dehydrogenase	3.21
<i>DIO1</i>	deiodinase, iodothyronine, type I	3.07
<i>TNFSF10</i>	tumor necrosis factor (ligand) superfamily, member 10 /// tumor necrosis factor (ligand) superfamily, member 10	2.96
<i>FABP1</i>	fatty acid binding protein 1, liver	2.85
<i>SERPINC1</i>	serpin peptidase inhibitor, clade C (antithrombin), member 1	2.83
<i>FGB</i>	fibrinogen beta chain	2.83
<i>HIST1H2AC</i>	histone cluster 1, H2ac	2.69
<i>AKR1D1</i>	aldo-keto reductase family 1, member D1 (delta 4-3-ketosteroid-5-beta-reductase)	2.67
<i>HIST1H4C</i>	histone cluster 1, H4c	2.61
<i>HIST1H3H</i>	histone cluster 1, H3h	2.55
<i>CYP4F12</i>	cytochrome P450, family 4, subfamily F, polypeptide 12	2.53
<i>HIST1H2BK</i>	histone cluster 1, H2bk	2.53
<i>ADH6</i>	alcohol dehydrogenase 6 (class V)	2.51
<i>RPL31</i> /// <i>LOC285260</i> /// <i>RPL31P4</i> /// <i>RPL31P10</i> /// <i>LOC641790</i> /// <i>LOC646841</i> /// <i>LOC648737</i> /// <i>LOC653773</i> /// <i>LOC727792</i> /// <i>LOC729646</i> /// <i>LOC732015</i>	ribosomal protein L31 /// similar to ribosomal protein L31 /// ribosomal protein L31 pseudogene 4 /// ribosomal protein L31 pseudogene 10 /// similar to ribosomal protein L31 /// similar to ribosomal protein L31 /// similar to ribosomal protein L31 /// similar to ribosomal protein L31 /// hypothetical protein LOC729646 /// similar to ribosomal protein L31	2.50
<i>RBKS</i>	ribokinase	2.44
<i>C4BPA</i>	complement component 4 binding protein, alpha	2.40
<i>TST</i>	thiosulfate sulfurtransferase (rhodanese)	2.39
<i>PGC</i>	progastricsin (pepsinogen C)	2.39
<i>NR1H4</i>	nuclear receptor subfamily 1, group H, member 4	2.37
<i>F11</i>	coagulation factor XI (plasma thromboplastin antecedent)	2.36
<i>HLA-DMB</i>	major histocompatibility complex, class II, DM beta /// major histocompatibility complex, class II, DM beta	2.33
<i>HAL</i>	histidine ammonia-lyase	2.31
<i>EHHADH</i>	enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	2.30
<i>TUBA3</i>	tubulin, alpha 3	2.29
<i>GHITM</i>	growth hormone inducible transmembrane protein	2.29
<i>C8B</i>	complement component 8, beta polypeptide	2.25
<i>HIST2H2AA3</i> /// <i>HIST2H2AA4</i>	histone cluster 2, H2aa3 /// histone cluster 2, H2aa4	2.24
<i>MAT1A</i>	methionine adenosyltransferase I, alpha	2.23
<i>CYP19A1</i>	cytochrome P450, family 19, subfamily A, polypeptide 1	2.20
<i>LGALS2</i>	lectin, galactoside-binding, soluble, 2 (galectin 2) /// lectin, galactoside-binding, soluble, 2 (galectin 2)	2.19
<i>AOC3</i>	amine oxidase, copper containing 3 (vascular adhesion protein 1)	2.18
<i>FGA</i>	fibrinogen alpha chain	2.18

Symbol	Gene name	Fold cahnge
<i>CASP4</i>	caspase 4, apoptosis-related cysteine peptidase	2.17
<i>RAE1</i>	RAE1 RNA export 1 homolog (S. pombe)	2.16
<i>NSDHL</i>	NAD(P) dependent steroid dehydrogenase-like	2.15
<i>MBL2</i>	mannose-binding lectin (protein C) 2, soluble (opsonic defect)	2.15
<i>LOC51136</i>	PTD016 protein	2.15
<i>PSMB9</i>	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	2.14
<i>SAA4</i>	serum amyloid A4, constitutive	2.13
<i>WWOX</i>	WW domain containing oxidoreductase	2.12
<i>HIST1H2BD</i>	histone cluster 1, H2bd	2.11
<i>ACSM3</i>	acyl-CoA synthetase medium-chain family member 3	2.11
<i>TFPI</i>	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	2.09
<i>POLR2G</i>	polymerase (RNA) II (DNA directed) polypeptide G	2.08
<i>SERPINA6</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 6	2.08
<i>SERPINA10</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 10	2.07
<i>PDPK1</i>	3-phosphoinositide dependent protein kinase-1	2.07
<i>ZFX</i>	zinc finger protein, X-linked	2.07
<i>PDZK1</i>	PDZ domain containing 1	2.07
<i>ALDH6A1</i>	aldehyde dehydrogenase 6 family, member A1	2.05
<i>GNG10</i> /// <i>LOC552891</i>	guanine nucleotide binding protein (G protein), gamma 10 /// hypothetical protein LOC552891	2.04
<i>DDC</i>	dopa decarboxylase (aromatic L-amino acid decarboxylase)	2.03
<i>CPN1</i>	carboxypeptidase N, polypeptide 1, 50kD	2.03
<i>HIST2H2BE</i>	histone cluster 2, H2be	2.02
<i>HMGCS1</i>	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	2.02
<i>HMGCL</i>	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase (hydroxymethylglutaricaciduria)	2.01
<i>CLGN</i>	calmegin	2.00
<i>TSPAN31</i>	tetraspanin 31	2.00

3 a) Up-regulated genes in 0.5 μ M exposure

Symbol	Gene name	Fold cahnge
<i>SLC2A3</i>	solute carrier family 2 (facilitated glucose transporter), member 3	16.12
<i>HMOX1</i>	heme oxygenase (decycling) 1	7.92
<i>CYR61</i>	cysteine-rich, angiogenic inducer, 61	7.32
<i>HAMP</i>	hepcidin antimicrobial peptide	5.01
<i>RBP1</i>	retinol binding protein 1, cellular	4.14
<i>FHL2</i>	four and a half LIM domains 2	3.97
<i>BHLHB2</i>	basic helix-loop-helix domain containing, class B, 2	3.61
<i>SERPINE1</i>	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	3.48
<i>KRT19</i>	keratin 19	3.36
<i>SFN</i>	stratifin	3.16
<i>FZD4</i>	frizzled homolog 4 (Drosophila)	3.15
<i>TPX2</i>	TPX2, microtubule-associated, homolog (Xenopus laevis)	3.07
	Transcribed locus, weakly similar to NP_001013229.1 rich protein 2 (predicted) [Rattus norvegicus]	3.02
<i>AURKB</i>	aurora kinase B	3.00
<i>WNK1</i>	WNK lysine deficient protein kinase 1 /// WNK lysine deficient protein kinase 1	2.95
<i>COTL1</i>	coactosin-like 1 (Dictyostelium)	2.89
<i>PTTG1</i>	pituitary tumor-transforming 1	2.82
<i>DKK1</i>	dickkopf homolog 1 (Xenopus laevis)	2.81
<i>CDC20</i>	cell division cycle 20 homolog (S. cerevisiae)	2.80
<i>SDPR</i>	serum deprivation response (phosphatidylserine binding protein)	2.77
<i>ADM</i>	adrenomedullin	2.77
<i>RPS6KA1</i>	ribosomal protein S6 kinase, 90kDa, polypeptide 1	2.76
<i>MCM2</i>	MCM2 minichromosome maintenance deficient 2, mitotin (S. cerevisiae)	2.75
<i>FYN</i>	FYN oncogene related to SRC, FGR, YES	2.74
<i>AKAP2</i> /// <i>PALM2-AKAP2</i>	A kinase (PRKA) anchor protein 2 /// PALM2-AKAP2 protein	2.74
<i>HEY1</i>	hair/enhancer-of-split related with YRPW motif 1	2.69
<i>ABCC1</i>	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	2.69
<i>CENPE</i>	centromere protein E, 312kDa	2.66
<i>BUB1B</i>	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	2.63
<i>CALR</i>	Calreticulin	2.59
<i>FLNA</i>	filamin A, alpha (actin binding protein 280)	2.55
<i>MFHAS1</i>	malignant fibrous histiocyoma amplified sequence 1	2.54
<i>HSPB1</i> /// <i>MEIS3</i>	heat shock 27kDa protein 1 /// Meis1, myeloid ecotropic viral integration site 1 homolog 3 (mouse)	2.54
<i>ID3</i>	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	2.54
<i>LRP8</i>	low density lipoprotein receptor-related protein 8, apolipoprotein e receptor	2.50
<i>AKR1B10</i>	aldo-keto reductase family 1, member B10 (aldose reductase)	2.48
<i>GPX3</i>	glutathione peroxidase 3 (plasma)	2.48
<i>AKAP12</i>	A kinase (PRKA) anchor protein (gravin) 12	2.48
<i>RAP2B</i>	RAP2B, member of RAS oncogene family	2.47
<i>SFN</i>	stratifin	2.46
<i>IER3</i>	immediate early response 3	2.46
<i>GCLM</i>	glutamate-cysteine ligase, modifier subunit	2.45
<i>MCCC2</i>	methylcrotonoyl-Coenzyme A carboxylase 2 (beta)	2.45
<i>TAGLN</i>	transgelin	2.44

Symbol	Gene name	Fold cahnge
<i>LGALS3</i>	lectin, galactoside-binding, soluble, 3 (galectin 3)	2.44
<i>CDC25B</i>	cell division cycle 25 homolog B (S. cerevisiae)	2.40
<i>RAI14</i>	retinoic acid induced 14	2.39
<i>ABCC3</i>	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	2.38
<i>SLC2A1</i>	solute carrier family 2 (facilitated glucose transporter), member 1	2.36
<i>TNFRSF21</i>	tumor necrosis factor receptor superfamily, member 21	2.32
<i>PMP22</i>	peripheral myelin protein 22	2.30
<i>KIF4A</i>	kinesin family member 4A	2.30
	CDNA FLJ34214 fis, clone FCBBF3021807	2.30
<i>CKAP4</i>	cytoskeleton-associated protein 4	2.27
<i>RRM2</i>	ribonucleotide reductase M2 polypeptide	2.27
<i>PIR</i>	pirin (iron-binding nuclear protein)	2.27
<i>SERPINB9</i>	serpin peptidase inhibitor, clade B (ovalbumin), member 9	2.25
<i>CRLF1</i>	cytokine receptor-like factor 1	2.24
<i>NDRG1</i>	N-myc downstream regulated gene 1	2.23
<i>IFI30</i>	interferon, gamma-inducible protein 30	2.22
<i>CSE1L</i>	CSE1 chromosome segregation 1-like (yeast)	2.22
<i>SNRPA</i>	small nuclear ribonucleoprotein polypeptide A	2.22
	Transcribed locus	2.21
<i>SOX4</i>	SRY (sex determining region Y)-box 4	2.21
<i>MRPS31</i>	mitochondrial ribosomal protein S31	2.20
<i>PPM1G</i>	protein phosphatase 1G (formerly 2C), magnesium-dependent, gamma isoform	2.20
<i>DUSP5</i>	dual specificity phosphatase 5	2.20
<i>CKB</i>	creatine kinase, brain	2.20
<i>TIMELESS</i>	timeless homolog (Drosophila)	2.19
<i>LASP1</i>	LIM and SH3 protein 1	2.18
<i>GATA2</i>	GATA binding protein 2	2.18
<i>RGS3</i>	regulator of G-protein signalling 3	2.15
<i>ANXA4</i>	annexin A4	2.14
<i>PRKCI</i>	protein kinase C, iota	2.14
<i>CKS2</i>	CDC28 protein kinase regulatory subunit 2	2.14
<i>PAK3 /// UBE2C</i>	p21 (CDKN1A)-activated kinase 3 /// ubiquitin-conjugating enzyme E2C	2.13
<i>PRC1</i>	protein regulator of cytokinesis 1	2.13
<i>TRIP13</i>	thyroid hormone receptor interactor 13	2.13
<i>CDKN3</i>	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	2.12
<i>TXNRD1</i>	thioredoxin reductase 1	2.11
<i>PKM2</i>	pyruvate kinase, muscle	2.11
<i>PRPF4</i>	PRP4 pre-mRNA processing factor 4 homolog (yeast)	2.11
<i>POLE2</i>	polymerase (DNA directed), epsilon 2 (p59 subunit)	2.11
<i>C1orf41 /// IL17RB</i>	chromosome 1 open reading frame 41 /// interleukin 17 receptor B	2.10
<i>PEA15</i>	phosphoprotein enriched in astrocytes 15	2.09
<i>ARMET</i>	arginine-rich, mutated in early stage tumors	2.09
<i>PIM1</i>	pim-1 oncogene /// pim-1 oncogene	2.07
<i>CENPF</i>	centromere protein F, 350/400ka (mitosin) /// centromere protein F, 350/400ka (mitosin)	2.07
<i>SRRM1</i>	serine/arginine repetitive matrix 1	2.06
<i>CPT1A</i>	carnitine palmitoyltransferase 1A (liver)	2.06
<i>NQO1</i>	NAD(P)H dehydrogenase, quinone 1	2.04

Symbol	Gene name	Fold cahnge
<i>KIF2C</i>	kinesin family member 2C	2.04
<i>BUB1</i>	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	2.03
		2.03
<i>NUSAP1</i>	nucleolar and spindle associated protein 1	2.03
<i>LMNA</i>	lamin A/C	2.03
<i>TPR /// BTRC</i>	translocated promoter region (to activated MET oncogene) /// beta-transducin repeat containing	2.00
<i>CYB5R4</i>	cytochrome b5 reductase 4	2.00

3 b) Down-regulated genes in 0.5 μ M exposure

Symbol	Gene name	Fold cahnge
<i>SLC17A2</i>	solute carrier family 17 (sodium phosphate), member 2	8.99
<i>AGTR1</i>	angiotensin II receptor, type 1	6.63
<i>NT5E</i>	5'-nucleotidase, ecto (CD73)	5.33
<i>TNFSF10</i>	tumor necrosis factor (ligand) superfamily, member 10 /// tumor necrosis factor (ligand) superfamily, member 10	5.19
<i>SERPINC1</i>	serpin peptidase inhibitor, clade C (antithrombin), member 1	4.97
<i>HIST1H2AC</i>	histone cluster 1, H2ac	4.96
<i>HIST1H4C</i>	histone cluster 1, H4c	4.78
<i>CLGN</i>	calmegin	4.48
<i>DIO1</i>	deiodinase, iodothyronine, type I	4.48
<i>FABP1</i>	fatty acid binding protein 1, liver	4.32
<i>CLU</i>	clusterin	3.91
<i>LGALS2</i>	lectin, galactoside-binding, soluble, 2 (galectin 2) /// lectin, galactoside-binding, soluble, 2 (galectin 2)	3.91
<i>STEAP1</i>	six transmembrane epithelial antigen of the prostate 1	3.82
<i>VTN</i>	vitronectin	3.81
<i>TFR2</i>	transferrin receptor 2	3.68
<i>RAD23B</i>	RAD23 homolog B (S. cerevisiae)	3.66
<i>MTTP</i>	microsomal triglyceride transfer protein	3.66
<i>CPN1</i>	carboxypeptidase N, polypeptide 1, 50kD	3.60
<i>IGSF1</i>	immunoglobulin superfamily, member 1	3.56
<i>PCK1</i>	phosphoenolpyruvate carboxykinase 1 (soluble)	3.51
<i>CIDEB</i>	cell death-inducing DFFA-like effector b	3.47
<i>F5</i>	coagulation factor V (proaccelerin, labile factor)	3.37
<i>ABCB4</i>	ATP-binding cassette, sub-family B (MDR/TAP), member 4	3.37
<i>FKBP11</i>	FK506 binding protein 11, 19 kDa	3.32
<i>IL1RAP</i>	interleukin 1 receptor accessory protein	3.27
<i>MATN3</i>	matrilin 3	3.16
<i>APOC3</i>	apolipoprotein C-III	3.15
<i>TSPAN7</i>	tetraspanin 7	3.13
<i>HIST2H2BE</i>	histone cluster 2, H2be	3.12
<i>SOAT2</i>	sterol O-acyltransferase 2	3.11
<i>SERPINI1</i>	serpin peptidase inhibitor, clade I (neuroserpin), member 1	3.10
<i>SERPINA4</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 4	3.09
<i>ITIH3</i>	inter-alpha (globulin) inhibitor H3	3.09
<i>SORBS1</i>	sorbin and SH3 domain containing 1	3.06
<i>AKR1D1</i>	aldo-keto reductase family 1, member D1 (delta 4-3-ketosteroid-5-beta-reductase)	2.99
<i>GLT8D1</i>	glycosyltransferase 8 domain containing 1	2.97
<i>TRAF4</i>	TNF receptor-associated factor 4	2.96
<i>POU2AF1</i>	POU domain, class 2, associating factor 1	2.96
<i>TTR</i>	transthyretin (prealbumin, amyloidosis type I)	2.95
<i>CES2</i>	carboxylesterase 2 (intestine, liver)	2.93
<i>SERPINA7</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7	2.84
<i>F11</i>	coagulation factor XI (plasma thromboplastin antecedent)	2.83
<i>PDZK1</i>	PDZ domain containing 1	2.80
<i>CCNT2</i>	cyclin T2	2.74
<i>PLEKHA1</i>	pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 1	2.72

Symbol	Gene name	Fold cahnge
<i>CYP51A1</i>	cytochrome P450, family 51, subfamily A, polypeptide 1	2.69
<i>STXBP3</i>	syntaxin binding protein 3	2.68
<i>ABCB1</i> /// <i>ABCB4</i>	ATP-binding cassette, sub-family B (MDR/TAP), member 1 /// ATP-binding cassette, sub-family B (MDR/TAP), member 4	2.66
<i>CPB2</i>	carboxypeptidase B2 (plasma, carboxypeptidase U)	2.65
<i>PSPH</i>	phosphoserine phosphatase	2.64
<i>AGXT</i>	alanine-glyoxylate aminotransferase (oxalosis I; hyperoxaluria I; glycolicaciduria; serine-pyruvate aminotransferase)	2.64
<i>LIPA</i>	lipase A, lysosomal acid, cholesterol esterase (Wolman disease)	2.63
<i>HPR</i>	haptoglobin-related protein	2.62
<i>BPHL</i>	biphenyl hydrolase-like (serine hydrolase; breast epithelial mucin-associated antigen)	2.60
<i>PDPK1</i>	3-phosphoinositide dependent protein kinase-1	2.57
<i>SERPINA5</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5	2.55
<i>F10</i>	coagulation factor X	2.54
<i>GPC3</i>	glypican 3	2.51
<i>PLSCR4</i>	phospholipid scramblase 4	2.51
<i>HAL</i>	histidine ammonia-lyase	2.50
<i>SERPINA6</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 6	2.49
<i>HIST2H2AA3</i> /// <i>HIST2H2AA4</i>	histone cluster 2, H2aa3 /// histone cluster 2, H2aa4	2.49
<i>UBD</i>	ubiquitin D	2.48
<i>NPM3</i>	nucleophosmin/nucleoplasmin, 3	2.45
<i>FRY</i>	furry homolog (Drosophila)	2.44
<i>PGRMC2</i>	progesterone receptor membrane component 2	2.42
<i>CLK1</i>	CDC-like kinase 1	2.41
<i>ZNF91</i>	zinc finger protein 91	2.41
<i>SLC1A2</i>	solute carrier family 1 (glial high affinity glutamate transporter), member 2	2.40
<i>CYP4F12</i>	cytochrome P450, family 4, subfamily F, polypeptide 12	2.38
<i>RAB17</i>	RAB17, member RAS oncogene family	2.37
<i>FGB</i>	fibrinogen beta chain	2.37
<i>TANK</i>	TRAF family member-associated NFKB activator	2.36
<i>MAGED2</i>	melanoma antigen family D, 2	2.36
<i>MBL2</i>	mannose-binding lectin (protein C) 2, soluble (opsonic defect)	2.36
<i>SAA4</i>	serum amyloid A4, constitutive	2.35
<i>ADH6</i>	alcohol dehydrogenase 6 (class V)	2.34
<i>TFPI</i>	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	2.33
<i>CD46</i>	CD46 molecule, complement regulatory protein	2.33
<i>NEK4</i>	NIMA (never in mitosis gene a)-related kinase 4	2.31
<i>DNAJC12</i>	DnaJ (Hsp40) homolog, subfamily C, member 12	2.30
<i>CLCN5</i>	chloride channel 5 (nephrolithiasis 2, X-linked, Dent disease)	2.30
<i>SERPINB1</i>	serpin peptidase inhibitor, clade B (ovalbumin), member 1	2.29
<i>C4BPA</i>	complement component 4 binding protein, alpha	2.29
<i>TST</i>	thiosulfate sulfurtransferase (rhodanese)	2.29
<i>BMI1</i>	B lymphoma Mo-MLV insertion region (mouse)	2.28
<i>C3orf9</i>	chromosome 3 open reading frame 9	2.26
<i>BCL6</i>	B-cell CLL/lymphoma 6 (zinc finger protein 51) /// B-cell CLL/lymphoma 6 (zinc finger protein 51)	2.25
<i>HIRA</i>	HIR histone cell cycle regulation defective homolog A (S. cerevisiae)	2.24
<i>TAF15</i>	TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 68kDa	2.24

Symbol	Gene name	Fold cahnge
<i>CPE</i>	carboxypeptidase E	2.23
<i>A2M</i>	alpha-2-macroglobulin	2.23
<i>C1RL</i>	complement component 1, r subcomponent-like	2.23
<i>PCOLCE2</i>	procollagen C-endopeptidase enhancer 2	2.23
<i>GAS2</i>	growth arrest-specific 2	2.21
<i>ABCA1</i>	ATP-binding cassette, sub-family A (ABC1), member 1	2.21
<i>SCML1</i>	sex comb on midleg-like 1 (Drosophila)	2.19
<i>APOA1</i>	apolipoprotein A-I	2.19
<i>ARSE</i>	arylsulfatase E (chondrodysplasia punctata 1)	2.19
<i>PROM1</i>	prominin 1	2.19
<i>TSC22D1</i>	TSC22 domain family, member 1	2.17
<i>PCYOX1</i>	prenylcysteine oxidase 1	2.17
<i>CCNG2</i>	cyclin G2	2.17
<i>IL6ST</i>	interleukin 6 signal transducer (gp130, oncostatin M receptor)	2.16
<i>SULT2A1</i>	sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1	2.16
<i>PECR</i>	peroxisomal trans-2-enoyl-CoA reductase	2.16
<i>USP18</i> /// <i>LOC727996</i> /// <i>LOC728216</i> /// <i>LOC728438</i>	ubiquitin specific peptidase 18 /// similar to ubiquitin specific peptidase 18 /// similar to ubiquitin specific peptidase 18	2.14
<i>SC4MOL</i>	sterol-C4-methyl oxidase-like	2.14
<i>GNG4</i>	guanine nucleotide binding protein (G protein), gamma 4	2.14
<i>ACOT2</i> /// <i>ACOT1</i>	acyl-CoA thioesterase 2 /// acyl-CoA thioesterase 1	2.12
<i>LGR4</i>	leucine-rich repeat-containing G protein-coupled receptor 4	2.12
<i>TGFBR3</i>	transforming growth factor, beta receptor III (betaglycan, 300kDa)	2.12
<i>CASP10</i>	caspase 10, apoptosis-related cysteine peptidase	2.11
<i>SLC38A3</i>	solute carrier family 38, member 3	2.11
<i>APOM</i>	apolipoprotein M	2.11
<i>BRP44L</i>	brain protein 44-like	2.10
<i>LIN7C</i>	lin-7 homolog C (C. elegans)	2.09
<i>COL4A5</i>	collagen, type IV, alpha 5 (Alport syndrome)	2.09
<i>C4A</i> /// <i>C4B</i>	complement component 4A (Rodgers blood group) /// complement component 4B (Childo blood group)	2.09
<i>EGR1</i>	early growth response 1	2.09
<i>ACP2</i>	acid phosphatase 2, lysosomal	2.08
<i>LAMP2</i>	lysosomal-associated membrane protein 2	2.08
<i>RAB40B</i>	RAB40B, member RAS oncogene family	2.08
<i>PURA</i>	purine-rich element binding protein A	2.07
<i>SERPINA10</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 10	2.07
<i>HIST1H1C</i>	histone cluster 1, H1c	2.07
<i>CA5A</i>	carbonic anhydrase VA, mitochondrial	2.07
<i>PDGFC</i>	platelet derived growth factor C	2.06
<i>PIGH</i>	phosphatidylinositol glycan anchor biosynthesis, class H	2.06
<i>NR2F1</i>	Nuclear receptor subfamily 2, group F, member 1	2.06
<i>CTH</i>	cystathionase (cystathionine gamma-lyase)	2.06
<i>AGT</i>	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	2.05
<i>HMGCR</i>	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	2.05
<i>INPP1</i>	inositol polyphosphate-1-phosphatase	2.05

Symbol	Gene name	Fold cahnge
<i>PGF</i>	placental growth factor, vascular endothelial growth factor-related protein	2.03
<i>RAB4A</i>	RAB4A, member RAS oncogene family	2.03
<i>ACVR1</i>	activin A receptor, type I	2.03
<i>IL17RB</i>	interleukin 17 receptor B	2.02
<i>PRODH2</i>	proline dehydrogenase (oxidase) 2	2.02
<i>ITPR2</i>	inositol 1,4,5-triphosphate receptor, type 2	2.01
<i>VPS4B</i>	vacuolar protein sorting 4 homolog B (S. cerevisiae)	2.01
<i>ACSL3</i>	acyl-CoA synthetase long-chain family member 3	2.01
<i>PSPH</i>	phosphoserine phosphatase	2.00

4 a) Up-regulated genes in 6 µM exposure

Symbol	Gene name	Fold cahnge
<i>HMOX1</i>	heme oxygenase (decycling) 1	66.94
<i>DUSP5</i>	dual specificity phosphatase 5	15.19
<i>CYR61</i>	cysteine-rich, angiogenic inducer, 61	10.80
<i>FHL2</i>	four and a half LIM domains 2	10.27
<i>MT1X</i>	metallothionein 1X	9.99
<i>TAGLN</i>	transgelin	9.11
<i>RBP1</i>	retinol binding protein 1, cellular	8.90
<i>HAMP</i>	hepcidin antimicrobial peptide	8.79
<i>PHLDA2</i>	pleckstrin homology-like domain, family A, member 2	7.50
<i>MT1X</i>	metallothionein 1X	7.08
<i>MT2A</i>	metallothionein 2A	6.51
<i>GCLM</i>	glutamate-cysteine ligase, modifier subunit	6.38
<i>GCNT3</i>	glucosaminyl (N-acetyl) transferase 3, mucin type	6.28
<i>MT1H</i>	metallothionein 1H	6.26
<i>PPP1R15A</i>	protein phosphatase 1, regulatory (inhibitor) subunit 15A	6.14
<i>HPCAL1</i>	hippocalcin-like 1	6.14
<i>HSPB1</i> /// <i>MEIS3</i>	heat shock 27kDa protein 1 /// Meis1, myeloid ecotropic viral integration site 1 homolog 3 (mouse)	5.76
<i>F2RL1</i>	coagulation factor II (thrombin) receptor-like 1	5.69
<i>CDC20</i>	cell division cycle 20 homolog (S. cerevisiae)	5.56
<i>SLC2A3</i>	solute carrier family 2 (facilitated glucose transporter), member 3	5.46
<i>TPM4</i>	tropomyosin 4	5.13
<i>ABCC1</i>	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	4.51
<i>HSPH1</i>	heat shock 105kDa/110kDa protein 1	4.46
<i>PTTG1</i>	pituitary tumor-transforming 1	4.29
<i>AKR1B10</i>	aldo-keto reductase family 1, member B10 (aldose reductase)	4.27
<i>PIR</i>	pirin (iron-binding nuclear protein)	4.25
<i>HSPA1A</i> /// <i>HSPA1B</i>	heat shock 70kDa protein 1A /// heat shock 70kDa protein 1B	4.18
<i>GBE1</i>	glucan (1,4-alpha-), branching enzyme 1 (glycogen branching enzyme, Andersen disease, glycogen storage disease type IV)	4.09
<i>CTSA</i>	cathepsin A	4.08
<i>SERPINE1</i>	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	3.97
<i>PLK1</i>	polo-like kinase 1 (Drosophila)	3.96
<i>TXNRD1</i>	thioredoxin reductase 1	3.91
<i>EMP3</i>	epithelial membrane protein 3	3.89
<i>TIMP1</i>	TIMP metalloproteinase inhibitor 1	3.84
<i>ZYX</i>	zyxin	3.81
<i>VAT1</i>	vesicle amine transport protein 1 homolog (T. californica)	3.78
<i>SFN</i>	stratifin	3.77
<i>NUPR1</i>	nuclear protein 1	3.73
<i>ALAS1</i>	aminolevulinate, delta-, synthase 1	3.70
<i>CTSL</i>	cathepsin L	3.69
<i>EPHX1</i>	epoxide hydrolase 1, microsomal (xenobiotic)	3.67
<i>BHMT2</i>	betaine-homocysteine methyltransferase 2	3.66
<i>AKR1B1</i>	aldo-keto reductase family 1, member B1 (aldose reductase)	3.65
<i>S100A10</i>	S100 calcium binding protein A10	3.63

Symbol	Gene name	Fold cahnge
<i>BLVRB</i>	biliverdin reductase B (flavin reductase (NADPH))	3.60
<i>LGALS1</i>	lectin, galactoside-binding, soluble, 1 (galectin 1)	3.58
<i>HGS</i>	hepatocyte growth factor-regulated tyrosine kinase substrate	3.56
<i>LGALS3</i>	lectin, galactoside-binding, soluble, 3 (galectin 3)	3.56
<i>STX3</i>	syntaxin 3	3.53
<i>TALDO1</i>	transaldolase 1	3.46
<i>RIT1</i>	Ras-like without CAAX 1	3.43
<i>CDKN3</i>	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	3.43
<i>PKM2</i>	pyruvate kinase, muscle	3.37
<i>KRT19</i>	keratin 19	3.37
<i>FOSL1</i>	FOS-like antigen 1	3.36
<i>ADM</i>	adrenomedullin	3.35
<i>FLNA</i>	filamin A, alpha (actin binding protein 280)	3.34
<i>UBE2S</i> /// <i>LOC731049</i>	ubiquitin-conjugating enzyme E2S /// similar to Ubiquitin-conjugating enzyme E2S (Ubiquitin-conjugating enzyme E2-24 kDa) (Ubiquitin-protein ligase) (Ubiquitin carrier protein) (E2-EPF5)	3.33
<i>PCQAP</i>	PC2 (positive cofactor 2, multiprotein complex) glutamine/Q-rich-associated protein	3.32
<i>HMGA1</i>	high mobility group AT-hook 1	3.31
<i>BUB1</i>	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	3.31
<i>CYB5R4</i>	cytochrome b5 reductase 4	3.25
<i>RAI14</i>	retinoic acid induced 14	3.21
<i>ATP6V1E1</i>	ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E1	3.17
<i>DRAP1</i>	DR1-associated protein 1 (negative cofactor 2 alpha)	3.16
<i>CDC25B</i>	cell division cycle 25 homolog B (S. cerevisiae)	3.15
<i>FECH</i>	ferrochelataase (protoporphyrin)	3.07
<i>NSFL1C</i>	NSFL1 (p97) cofactor (p47)	3.07
<i>GSR</i>	glutathione reductase	3.07
<i>CCNA2</i>	cyclin A2	3.04
<i>TPX2</i>	TPX2, microtubule-associated, homolog (Xenopus laevis)	3.03
<i>SFN</i>	stratifin	2.97
<i>IFI30</i>	interferon, gamma-inducible protein 30	2.96
<i>CDKN1A</i>	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	2.94
<i>DDX39</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39	2.94
<i>PRC1</i>	protein regulator of cytokinesis 1	2.93
<i>TRIM16</i> /// <i>TRIM16L</i>	tripartite motif-containing 16 /// tripartite motif-containing 16-like	2.93
<i>KYNU</i>	kynureninase (L-kynurenine hydrolase)	2.92
<i>ANXA3</i>	annexin A3	2.92
<i>PEA15</i>	phosphoprotein enriched in astrocytes 15	2.90
<i>KNS2</i>	kinesin 2	2.86
<i>CD58</i>	CD58 molecule	2.85
<i>KIF2C</i>	kinesin family member 2C	2.85
<i>GADD45B</i>	growth arrest and DNA-damage-inducible, beta	2.84
<i>TGFB1</i>	transforming growth factor, beta 1 (Camurati-Engelmann disease)	2.83
<i>RRM2</i>	ribonucleotide reductase M2 polypeptide	2.83
<i>SERPINH1</i>	serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	2.82
<i>BUB1B</i>	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	2.81
<i>GRB10</i>	growth factor receptor-bound protein 10	2.80

Symbol	Gene name	Fold cahnge
<i>PLK2</i>	polo-like kinase 2 (Drosophila)	2.79
<i>LRP8</i>	low density lipoprotein receptor-related protein 8, apolipoprotein e receptor	2.79
<i>ADRM1</i>	adhesion regulating molecule 1	2.77
<i>AKAP12</i>	A kinase (PRKA) anchor protein (gravin) 12	2.76
<i>UCHL3</i>	ubiquitin carboxyl-terminal esterase L3 (ubiquitin thiolesterase)	2.75
<i>CAPN2</i>	calpain 2, (m/II) large subunit	2.75
<i>LMNA</i>	lamin A/C	2.74
<i>MVP</i>	major vault protein	2.74
<i>DDEF2</i>	development and differentiation enhancing factor 2	2.74
<i>PSMD14</i>	proteasome (prosome, macropain) 26S subunit, non-ATPase, 14	2.73
<i>DTNA</i>	dystrobrevin, alpha	2.71
<i>TUBB3</i>	tubulin, beta 3	2.70
<i>RPS6KA1</i>	ribosomal protein S6 kinase, 90kDa, polypeptide 1	2.68
<i>PSMD3</i>	proteasome (prosome, macropain) 26S subunit, non-ATPase, 3	2.67
<i>POLR3C</i>	polymerase (RNA) III (DNA directed) polypeptide C (62kD)	2.66
<i>SNRPA</i>	small nuclear ribonucleoprotein polypeptide A	2.63
<i>PTOV1</i>	prostate tumor overexpressed gene 1	2.62
<i>PMP22</i>	peripheral myelin protein 22	2.60
<i>RGS3</i>	regulator of G-protein signalling 3	2.60
<i>RHOC</i>	ras homolog gene family, member C	2.60
<i>RNF141</i>	ring finger protein 141	2.59
<i>ASNA1</i>	arsA arsenite transporter, ATP-binding, homolog 1 (bacterial)	2.59
<i>ABCC2</i>	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	2.59
<i>SQSTM1</i>	sequestosome 1	2.59
<i>STK17A</i>	serine/threonine kinase 17a (apoptosis-inducing)	2.57
<i>EHMT2</i>	euchromatic histone-lysine N-methyltransferase 2	2.56
<i>CALR</i>	Calreticulin	2.56
<i>TOMM34</i>	translocase of outer mitochondrial membrane 34	2.55
<i>PSMD2</i>	proteasome (prosome, macropain) 26S subunit, non-ATPase, 2	2.55
<i>IER3</i>	immediate early response 3	2.55
<i>GLA</i>	galactosidase, alpha	2.55
<i>KIF20A</i>	kinesin family member 20A	2.54
<i>C20orf24</i>	chromosome 20 open reading frame 24	2.54
<i>NP</i>	nucleoside phosphorylase	2.53
<i>M6PRBP1</i>	mannose-6-phosphate receptor binding protein 1	2.53
<i>STIP1</i>	stress-induced-phosphoprotein 1 (Hsp70/Hsp90-organizing protein)	2.52
<i>GNPDA1</i>	glucosamine-6-phosphate deaminase 1	2.52
<i>HN1</i>	hematological and neurological expressed 1	2.51
<i>DRG2</i>	developmentally regulated GTP binding protein 2	2.51
<i>ST6GALNAC4</i>	ST6 (alpha-N-acetyl-neuraminy-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 4	2.51
<i>PITPNC1</i>	phosphatidylinositol transfer protein, cytoplasmic 1	2.50
<i>VRK2</i>	vaccinia related kinase 2	2.49
<i>EXOSC10</i>	exosome component 10	2.48
<i>ECD</i>	ecdysoneless homolog (Drosophila)	2.48
<i>SERPINE2</i>	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2	2.48
<i>FKBP4</i>	FK506 binding protein 4, 59kDa	2.47
<i>CCNB2</i>	cyclin B2	2.47

Symbol	Gene name	Fold cahnge
<i>RANGAP1</i>	Ran GTPase activating protein 1	2.42
<i>HSPA4L</i>	heat shock 70kDa protein 4-like	2.41
<i>CORO1C</i>	coronin, actin binding protein, 1C	2.41
<i>MAP2K2</i>	mitogen-activated protein kinase kinase 2	2.41
<i>PIK3CD</i>	phosphoinositide-3-kinase, catalytic, delta polypeptide /// phosphoinositide-3-kinase, catalytic, delta polypeptide	2.40
<i>NUP155</i>	nucleoporin 155kDa	2.39
<i>AHSA1</i>	AHA1, activator of heat shock 90kDa protein ATPase homolog 1 (yeast)	2.39
<i>KIF4A</i>	kinesin family member 4A	2.39
<i>NQO2</i>	NAD(P)H dehydrogenase, quinone 2	2.39
<i>GNB1L</i>	guanine nucleotide binding protein (G protein), beta polypeptide 1-like	2.38
<i>VIL2</i>	villin 2 (ezrin)	2.38
<i>ATP6V1C1</i>	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1	2.37
<i>COTL1</i>	coactosin-like 1 (Dictyostelium)	2.37
<i>FAF1</i>	Fas (TNFRSF6) associated factor 1	2.36
<i>USP11</i>	ubiquitin specific peptidase 11	2.36
<i>NUSAP1</i>	nucleolar and spindle associated protein 1	2.35
<i>PAK3 /// UBE2C</i>	p21 (CDKN1A)-activated kinase 3 /// ubiquitin-conjugating enzyme E2C	2.35
<i>PNKP</i>	polynucleotide kinase 3'-phosphatase	2.35
<i>CAP2</i>	CAP, adenylate cyclase-associated protein, 2 (yeast)	2.35
<i>YKT6</i>	YKT6 v-SNARE homolog (S. cerevisiae)	2.34
<i>MGAT4B</i>	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isozyme B	2.34
<i>BAG2</i>	BCL2-associated athanogene 2	2.33
<i>MDK</i>	midkine (neurite growth-promoting factor 2)	2.32
<i>MAFF</i>	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	2.31
<i>NMB</i>	neuromedin B	2.31
<i>TUBB2C</i>	tubulin, beta 2C	2.31
<i>AK1</i>	adenylate kinase 1	2.31
<i>THOP1</i>	thimet oligopeptidase 1	2.30
<i>SHB</i>	Src homology 2 domain containing adaptor protein B	2.29
<i>PTPN3</i>	protein tyrosine phosphatase, non-receptor type 3	2.28
<i>DDX56</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 56	2.28
<i>ARPC4</i>	actin related protein 2/3 complex, subunit 4, 20kDa	2.28
<i>AVEN</i>	apoptosis, caspase activation inhibitor	2.26
<i>TAX1BP1</i>	Tax1 (human T-cell leukemia virus type I) binding protein 1	2.26
<i>C1S</i>	complement component 1, s subcomponent	2.24
<i>FLII</i>	flightless I homolog (Drosophila)	2.24
<i>CHIC2</i>	cysteine-rich hydrophobic domain 2	2.23
<i>GRSF1</i>	G-rich RNA sequence binding factor 1	2.23
<i>CKS1B</i>	CDC28 protein kinase regulatory subunit 1B	2.23
<i>CKS2</i>	CDC28 protein kinase regulatory subunit 2	2.21
<i>GYS1</i>	glycogen synthase 1 (muscle)	2.21
<i>RHOQ</i>	ras homolog gene family, member Q	2.21
<i>TMSB10</i>	thymosin, beta 10	2.21
<i>PHTF1</i>	putative homeodomain transcription factor 1	2.20
<i>SPAG5</i>	sperm associated antigen 5	2.20
<i>BANF1</i>	barrier to autointegration factor 1	2.19
<i>MPHOSPH1</i>	M-phase phosphoprotein 1	2.19

Symbol	Gene name	Fold cahnge
<i>PSMD1</i>	proteasome (prosome, macropain) 26S subunit, non-ATPase, 1	2.19
<i>SDF2L1</i>	stromal cell-derived factor 2-like 1	2.18
<i>BHLHB2</i>	basic helix-loop-helix domain containing, class B, 2	2.17
<i>CENPE</i>	centromere protein E, 312kDa	2.17
<i>BIRC5</i>	baculoviral IAP repeat-containing 5 (survivin)	2.17
<i>MYO1E</i> /// <i>LOC390588</i>	myosin IE /// similar to CDK105 protein	2.16
<i>GATA2</i>	GATA binding protein 2	2.15
<i>TMEM111</i>	transmembrane protein 111	2.14
<i>NCAPG</i>	non-SMC condensin I complex, subunit G	2.14
<i>WNK1</i>	WNK lysine deficient protein kinase 1 /// WNK lysine deficient protein kinase 1	2.14
<i>TLE3</i>	transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)	2.13
<i>SH3BP4</i>	SH3-domain binding protein 4	2.12
<i>ATF5</i>	activating transcription factor 5	2.12
<i>PTBP1</i>	polypyrimidine tract binding protein 1	2.12
<i>SNN</i>	stannin	2.12
<i>SLC20A1</i>	solute carrier family 20 (phosphate transporter), member 1	2.11
<i>SMARCA2</i>	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2	2.11
<i>TSC2</i>	tuberous sclerosis 2	2.11
<i>TRIP12</i>	thyroid hormone receptor interactor 12	2.10
<i>NAPA</i>	N-ethylmaleimide-sensitive factor attachment protein, alpha	2.10
<i>BAG3</i>	BCL2-associated athanogene 3	2.10
<i>PPME1</i>	protein phosphatase methylesterase 1	2.10
<i>RPP30</i>	ribonuclease P/MRP 30kDa subunit	2.09
<i>MAP1LC3B</i>	microtubule-associated protein 1 light chain 3 beta	2.09
<i>FGFR1OP</i> /// <i>C9orf4</i>	FGFR1 oncogene partner /// chromosome 9 open reading frame 4	2.09
<i>KPNA6</i>	karyopherin alpha 6 (importin alpha 7)	2.08
<i>GSTO1</i>	glutathione S-transferase omega 1	2.08
<i>KIF23</i>	kinesin family member 23	2.07
<i>ZNF259</i> /// <i>LOC442240</i>	zinc finger protein 259 /// zinc finger protein 259 /// similar to zinc finger protein 259 /// similar to zinc finger protein 259	2.06
<i>MKLN1</i>	muskelin 1, intracellular mediator containing kelch motifs	2.05
<i>ACTR1A</i>	ARP1 actin-related protein 1 homolog A, contractin alpha (yeast)	2.05
<i>FUBP1</i>	far upstream element (FUSE) binding protein 1	2.04
<i>UVRAG</i>	UV radiation resistance associated gene	2.04
<i>TOMM22</i>	translocase of outer mitochondrial membrane 22 homolog (yeast)	2.03
<i>FAH</i>	fumarylacetoacetate hydrolase (fumarylacetoacetase)	2.03
<i>PSMD12</i>	proteasome (prosome, macropain) 26S subunit, non-ATPase, 12	2.03
<i>AURKA</i>	aurora kinase A	2.02
<i>PSMB2</i>	proteasome (prosome, macropain) subunit, beta type, 2 /// proteasome (prosome, macropain) subunit, beta type, 2	2.02
<i>ALDH1L1</i>	aldehyde dehydrogenase 1 family, member L1	2.02
<i>FABP5</i> /// <i>LOC728641</i> /// <i>LOC729163</i> /// <i>LOC731043</i> /// <i>LOC732031</i>	fatty acid binding protein 5 (psoriasis-associated) /// similar to Fatty acid-binding protein, epidermal (E-FABP) (Psoriasis-associated fatty acid-binding protein homolog) (PA-FABP) /// similar to Fatty acid-binding protein, epidermal (E-FABP) (Psoriasis-associated fatty acid-binding protein homolog) (PA-FABP) /// similar to Fatty acid-binding protein, epidermal (E-FABP) (Psoriasis-associated fatty acid-binding protein homolog) (PA-FABP) /// similar to Fatty acid-binding protein, epidermal (E-FABP) (Psoriasis-associated fatty acid-binding protein homolog) (PA-FABP)	2.01

Symbol	Gene name	Fold cahnge
<i>ATG5</i>	ATG5 autophagy related 5 homolog (S. cerevisiae)	2.01
<i>SQRDL</i>	sulfide quinone reductase-like (yeast)	2.00

4 b) Down-regulated genes in 6 µM exposure

Symbol	Gene name	Fold cahnge
<i>FABP1</i>	fatty acid binding protein 1, liver	42.75
<i>TGFBR3</i>	transforming growth factor, beta receptor III (betaglycan, 300kDa)	28.05
<i>SERPINA7</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7	17.91
<i>SC4MOL</i>	sterol-C4-methyl oxidase-like	11.82
<i>CCL20</i>	chemokine (C-C motif) ligand 20	9.92
<i>CIDEB</i>	cell death-inducing DFFA-like effector b	9.82
<i>NR1H4</i>	nuclear receptor subfamily 1, group H, member 4	8.36
<i>CPB2</i>	carboxypeptidase B2 (plasma, carboxypeptidase U)	8.19
<i>PGC</i>	progastricsin (pepsinogen C)	8.01
<i>UBD</i>	ubiquitin D	7.86
<i>MBL2</i>	mannose-binding lectin (protein C) 2, soluble (opsonic defect)	7.75
<i>SERPINB1</i>	serpin peptidase inhibitor, clade B (ovalbumin), member 1	7.62
<i>ADH6</i>	alcohol dehydrogenase 6 (class V)	7.54
<i>TNFSF10</i>	tumor necrosis factor (ligand) superfamily, member 10 /// tumor necrosis factor (ligand) superfamily, member 10	6.56
<i>FMO5</i>	flavin containing monooxygenase 5	6.43
<i>VSNL1</i>	visinin-like 1	6.35
<i>TNFAIP3</i>	tumor necrosis factor, alpha-induced protein 3	6.25
<i>P2RY5</i>	purinergic receptor P2Y, G-protein coupled, 5	6.16
<i>SERPINA5</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5	6.11
<i>HIST1H4C</i>	histone cluster 1, H4c	6.01
<i>HMGS1</i>	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	5.82
<i>APOC3</i>	apolipoprotein C-III	5.81
<i>B3GNT1</i>	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1	5.73
<i>ANG /// RNASE4</i>	angiogenin, ribonuclease, RNase A family, 5 /// ribonuclease, RNase A family, 4	5.60
<i>SQLE</i>	squalene epoxidase	5.51
<i>CFB</i>	complement factor B	5.47
<i>DHCR7</i>	7-dehydrocholesterol reductase	5.39
<i>MTTP</i>	microsomal triglyceride transfer protein	5.38
<i>NT5E</i>	5'-nucleotidase, ecto (CD73)	5.35
<i>SOAT2</i>	sterol O-acyltransferase 2	5.29
<i>HMGCR</i>	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	5.28
<i>POU2AF1</i>	POU domain, class 2, associating factor 1	5.24
<i>ZNF217</i>	zinc finger protein 217	5.19
<i>SERPINA6</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 6	5.18
<i>LGR4</i>	leucine-rich repeat-containing G protein-coupled receptor 4	5.16
<i>SEPP1</i>	selenoprotein P, plasma, 1	5.12
<i>RNASE4</i>	ribonuclease, RNase A family, 4	5.11
<i>AOC3</i>	amine oxidase, copper containing 3 (vascular adhesion protein 1)	5.02
<i>NEDD9</i>	neural precursor cell expressed, developmentally down-regulated 9	5.01
<i>CP</i>	ceruloplasmin (ferroxidase)	4.95
<i>CPN1</i>	carboxypeptidase N, polypeptide 1, 50kD	4.89
<i>ACSL3</i>	acyl-CoA synthetase long-chain family member 3	4.74
<i>SGK</i>	serum/glucocorticoid regulated kinase	4.67
<i>ALCAM</i>	activated leukocyte cell adhesion molecule	4.61
<i>CLGN</i>	calmegin	4.58
<i>IGSF4</i>	immunoglobulin superfamily, member 4	4.54

Symbol	Gene name	Fold cahnge
<i>SLC17A2</i>	solute carrier family 17 (sodium phosphate), member 2	4.54
<i>HOXD1</i>	homeobox D1	4.53
<i>RAPGEF5</i>	Rap guanine nucleotide exchange factor (GEF) 5	4.47
<i>TTR</i>	transthyretin (prealbumin, amyloidosis type I)	4.41
<i>PCYOX1</i>	prenylcysteine oxidase 1	4.36
<i>NRIP1</i>	nuclear receptor interacting protein 1	4.36
<i>PER2</i>	period homolog 2 (Drosophila)	4.36
<i>F5</i>	coagulation factor V (proaccelerin, labile factor)	4.34
<i>LIPA</i>	lipase A, lysosomal acid, cholesterol esterase (Wolman disease)	4.26
<i>SERPINA3</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	4.13
<i>TST</i>	thiosulfate sulfurtransferase (rhodanese)	4.12
<i>ENC1</i>	ectodermal-neural cortex (with BTB-like domain)	4.11
<i>RGN</i>	regucalcin (senescence marker protein-30)	4.10
<i>PGRMC2</i>	progesterone receptor membrane component 2	4.00
<i>IGSF1</i>	immunoglobulin superfamily, member 1	4.00
<i>MATN3</i>	matrilin 3	3.98
<i>SOX9</i>	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	3.96
<i>IL1R1</i>	interleukin 1 receptor, type I	3.95
<i>PIP5K1B</i>	phosphatidylinositol-4-phosphate 5-kinase, type I, beta	3.93
<i>ABCB4</i>	ATP-binding cassette, sub-family B (MDR/TAP), member 4	3.92
<i>FOXA2</i>	forkhead box A2	3.92
<i>APOM</i>	apolipoprotein M	3.89
<i>FGFR3</i>	fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)	3.89
<i>AGT</i>	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	3.88
<i>C14orf1</i>	chromosome 14 open reading frame 1	3.88
<i>ITPR2</i>	inositol 1,4,5-triphosphate receptor, type 2	3.81
<i>LOX</i>	lysyl oxidase	3.80
<i>ASGR1</i>	asialoglycoprotein receptor 1	3.72
<i>DUSP6</i>	dual specificity phosphatase 6	3.72
<i>ATP7B</i>	ATPase, Cu++ transporting, beta polypeptide	3.63
<i>C4BPB</i>	complement component 4 binding protein, beta	3.63
<i>CTGF</i>	connective tissue growth factor	3.62
<i>BRP44L</i>	brain protein 44-like	3.61
<i>ARSE</i>	arylsulfatase E (chondrodysplasia punctata 1)	3.60
<i>SERPINC1</i>	serpin peptidase inhibitor, clade C (antithrombin), member 1	3.58
<i>ACOT2 /// ACOT1</i>	acyl-CoA thioesterase 2 /// acyl-CoA thioesterase 1	3.55
<i>ITI1H3</i>	inter-alpha (globulin) inhibitor H3	3.52
<i>SC5DL</i>	sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, fungal)-like	3.52
<i>TRIM15</i>	tripartite motif-containing 15	3.47
<i>HYAL1</i>	hyaluronoglucosaminidase 1	3.44
<i>GBAS</i>	glioblastoma amplified sequence	3.43
<i>DICER1</i>	Dicer1, Dcr-1 homolog (Drosophila)	3.42
<i>ARL4D</i>	ADP-ribosylation factor-like 4D	3.41
<i>SLC39A7</i>	solute carrier family 39 (zinc transporter), member 7	3.38
<i>ZFP36L1</i>	zinc finger protein 36, C3H type-like 1	3.34
<i>PROM1</i>	prominin 1	3.32
<i>IDI1</i>	isopentenyl-diphosphate delta isomerase 1	3.32
<i>TFPI</i>	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	3.31

Symbol	Gene name	Fold cahnge
<i>CD46</i>	CD46 molecule, complement regulatory protein	3.30
<i>F11</i>	coagulation factor XI (plasma thromboplastin antecedent)	3.29
<i>AKR1D1</i>	aldo-keto reductase family 1, member D1 (delta 4-3-ketosteroid-5-beta-reductase)	3.27
<i>FDPS</i>	farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyltranstransferase, geranyltranstransferase)	3.25
<i>APOA1</i>	apolipoprotein A-I	3.23
<i>PLLP</i>	plasma membrane proteolipid (plasmolipin)	3.22
<i>MGAT2</i>	mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase	3.21
<i>CPE</i>	carboxypeptidase E	3.20
<i>PCNP</i>	PEST proteolytic signal containing nuclear protein	3.18
<i>PSPH</i>	phosphoserine phosphatase	3.17
<i>TACSTD1</i>	tumor-associated calcium signal transducer 1	3.14
<i>DNAJC12</i>	DnaJ (Hsp40) homolog, subfamily C, member 12	3.12
<i>SLC2A10</i>	solute carrier family 2 (facilitated glucose transporter), member 10 /// solute carrier family 2 (facilitated glucose transporter), member 10	3.11
<i>SCML1</i>	sex comb on midleg-like 1 (Drosophila)	3.09
<i>SERPIND1</i>	serpin peptidase inhibitor, clade D (heparin cofactor), member 1	3.09
<i>RABGGTB</i>	Rab geranylgeranyltransferase, beta subunit	3.04
<i>PURA</i>	purine-rich element binding protein A	3.02
<i>ANPEP</i>	alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)	3.02
<i>FGA</i>	fibrinogen alpha chain	3.01
<i>NFAT5</i>	nuclear factor of activated T-cells 5, tonicity-responsive	3.00
<i>IL17RB</i>	interleukin 17 receptor B	3.00
<i>CA5A</i>	carbonic anhydrase VA, mitochondrial	3.00
<i>GJB1</i>	gap junction protein, beta 1, 32kDa (connexin 32, Charcot-Marie-Tooth neuropathy, X-linked)	2.96
<i>HIST1H2AC</i>	histone cluster 1, H2ac	2.95
<i>DLEU1</i> /// <i>SPANXC</i>	deleted in lymphocytic leukemia, 1 /// SPANX family, member C	2.92
<i>SCD</i>	stearoyl-CoA desaturase (delta-9-desaturase)	2.91
<i>RPL31</i> /// <i>LOC285260</i> /// <i>RPL31P4</i> /// <i>RPL31P10</i> /// <i>LOC641790</i> /// <i>LOC646841</i> /// <i>LOC648737</i> /// <i>LOC653773</i> /// <i>LOC727792</i> /// <i>LOC729646</i> /// <i>LOC732015</i>	ribosomal protein L31 /// similar to ribosomal protein L31 /// ribosomal protein L31 pseudogene 4 /// ribosomal protein L31 pseudogene 10 /// similar to ribosomal protein L31 /// similar to ribosomal protein L31 /// similar to ribosomal protein L31 /// similar to ribosomal protein L31 /// hypothetical protein LOC729646 /// similar to ribosomal protein L31	2.90
<i>TSC22D1</i>	TSC22 domain family, member 1	2.90
<i>ZNF318</i>	zinc finger protein 318	2.89
<i>SLC31A1</i>	solute carrier family 31 (copper transporters), member 1	2.88
<i>MAGED1</i>	melanoma antigen family D, 1	2.87
<i>MAP4K3</i>	mitogen-activated protein kinase kinase kinase kinase 3	2.85
<i>SLC35E3</i>	solute carrier family 35, member E3	2.84
<i>SPHAR</i>	S-phase response (cyclin-related)	2.84
<i>TSPAN8</i>	tetraspanin 8	2.83
<i>ACADM</i>	acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain	2.82

Symbol	Gene name	Fold cahnge
<i>PLOD2</i>	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2	2.81
<i>CFI</i>	complement factor I	2.81
<i>ZNF91</i>	zinc finger protein 91	2.80
<i>PTCH1</i>	patched homolog 1 (Drosophila)	2.80
<i>AZGP1</i>	alpha-2-glycoprotein 1, zinc-binding	2.80
<i>TSPAN6</i>	tetraspanin 6	2.79
<i>SULT2A1</i>	sulfotransferase family, cytosolic, 2A, dehydroepiandrosterone (DHEA)-preferring, member 1	2.79
<i>MMD</i>	monocyte to macrophage differentiation-associated	2.78
<i>VTN</i>	vitronectin	2.78
<i>ZNF331</i>	zinc finger protein 331	2.77
<i>CPD</i>	carboxypeptidase D	2.77
<i>BTN3A3</i> /// <i>BTN3A2</i>	butyrophilin, subfamily 3, member A3 /// butyrophilin, subfamily 3, member A2	2.75
<i>KTN1</i>	kinectin 1 (kinesin receptor)	2.75
<i>XBP1</i>	X-box binding protein 1	2.74
<i>F10</i>	coagulation factor X	2.73
<i>HIRA</i>	HIR histone cell cycle regulation defective homolog A (S. cerevisiae)	2.72
<i>SERPINF2</i>	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 2	2.71
<i>COX11</i>	COX11 homolog, cytochrome c oxidase assembly protein (yeast)	2.71
<i>PLSCR1</i>	phospholipid scramblase 1	2.71
<i>EBAG9</i>	estrogen receptor binding site associated, antigen, 9	2.70
		2.70
<i>C10orf10</i>	chromosome 10 open reading frame 10	2.69
<i>MERTK</i>	c-mer proto-oncogene tyrosine kinase	2.68
<i>FDFT1</i>	farnesyl-diphosphate farnesyltransferase 1	2.68
<i>TTC3</i>	tetratricopeptide repeat domain 3	2.68
<i>RAB4A</i>	RAB4A, member RAS oncogene family	2.68
<i>SGCE</i>	sarcoglycan, epsilon	2.68
<i>ABCA1</i>	ATP-binding cassette, sub-family A (ABC1), member 1	2.68
<i>AMACR</i>	alpha-methylacyl-CoA racemase	2.67
<i>FILIP1L</i>	filamin A interacting protein 1-like	2.67
<i>SLC2A4RG</i>	SLC2A4 regulator	2.65
<i>HLF</i>	hepatic leukemia factor	2.65
<i>SFRS2IP</i>	splicing factor, arginine/serine-rich 2, interacting protein	2.64
<i>NET1</i>	neuroepithelial cell transforming gene 1	2.64
<i>TACC1</i>	transforming, acidic coiled-coil containing protein 1	2.64
<i>TIA1</i>	TIA1 cytotoxic granule-associated RNA binding protein	2.64
<i>LEPRE1</i>	leucine proline-enriched proteoglycan (leprecan) 1	2.61
<i>RELN</i>	reelin	2.61
<i>SLC37A4</i>	solute carrier family 37 (glycerol-6-phosphate transporter), member 4	2.60
<i>EFNA1</i>	ephrin-A1	2.59
<i>PSPH</i>	phosphoserine phosphatase	2.58
<i>BMI1</i>	B lymphoma Mo-MLV insertion region (mouse)	2.57
<i>CETN3</i>	centrin, EF-hand protein, 3 (CDC31 homolog, yeast)	2.57
<i>LOC653879</i>	similar to Complement C3 precursor	2.57
<i>DHCR24</i>	24-dehydrocholesterol reductase	2.56
<i>C4BPA</i>	complement component 4 binding protein, alpha	2.56

Symbol	Gene name	Fold cahnge
<i>CCNI</i>	Cyclin I	2.56
<i>LGR5</i>	leucine-rich repeat-containing G protein-coupled receptor 5	2.56
<i>FGB</i>	fibrinogen beta chain	2.55
<i>ITGAV</i>	integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)	2.55
<i>PTPRK</i>	protein tyrosine phosphatase, receptor type, K	2.52
<i>RHOB</i>	ras homolog gene family, member B	2.52
<i>RBMS1</i>	RNA binding motif, single stranded interacting protein 1	2.52
<i>HMGNA</i>	high mobility group nucleosomal binding domain 4	2.51
<i>NDUFA5</i>	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kDa	2.50
<i>FKBP1B</i>	FK506 binding protein 1B, 12.6 kDa	2.50
<i>BAX</i>	BCL2-associated X protein	2.50
<i>IKBKAP</i>	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	2.50
<i>HNMT</i>	histamine N-methyltransferase	2.50
<i>IGF2 /// INS-IGF2</i>	insulin-like growth factor 2 (somatomedin A) /// insulin- insulin-like growth factor 2	2.49
<i>A2M</i>	alpha-2-macroglobulin	2.49
<i>SORL1</i>	sortilin-related receptor, L(DLR class) A repeats-containing	2.48
<i>AHR</i>	aryl hydrocarbon receptor	2.47
<i>FBXL4</i>	F-box and leucine-rich repeat protein 4	2.45
<i>2-Sep</i>	septin 2 /// septin 2	2.45
<i>PAH</i>	phenylalanine hydroxylase	2.45
<i>CLDN1</i>	claudin 1	2.45
<i>C2orf28</i>	chromosome 2 open reading frame 28	2.45
<i>DGAT1 /// LOC727765</i>	diacylglycerol O-acyltransferase homolog 1 (mouse) /// similar to Diacylglycerol O-acyltransferase 1 (Diglyceride acyltransferase) (ACAT-related gene product 1)	2.44
<i>NAP1L1</i>	nucleosome assembly protein 1-like 1	2.42
<i>ENSA</i>	endosulfine alpha	2.41
<i>NEK4</i>	NIMA (never in mitosis gene a)-related kinase 4	2.41
<i>SLC1A2</i>	solute carrier family 1 (glial high affinity glutamate transporter), member 2	2.40
<i>HHEX</i>	homeobox, hematopoietically expressed	2.40
<i>GSN</i>	gelsolin (amyloidosis, Finnish type)	2.40
<i>RPL31</i>	ribosomal protein L31	2.40
<i>ACAA2</i>	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	2.39
<i>SLC2A2</i>	solute carrier family 2 (facilitated glucose transporter), member 2	2.38
<i>KRIT1</i>	KRIT1, ankyrin repeat containing	2.38
<i>LOC730013</i>	similar to ATP-binding cassette, sub-family C, member 6	2.38
<i>ICAM1</i>	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	2.38
<i>HMGCL</i>	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase (hydroxymethylglutaricaciduria)	2.38
<i>H1FO</i>	H1 histone family, member 0	2.38
<i>ERBB2</i>	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	2.38
<i>GAS2</i>	growth arrest-specific 2	2.37
<i>CYP3A7</i>	cytochrome P450, family 3, subfamily A, polypeptide 7	2.34
<i>PCM1 /// TPTE</i>	pericentriolar material 1 /// transmembrane phosphatase with tensin homology	2.34
<i>EIF2AK3</i>	eukaryotic translation initiation factor 2-alpha kinase 3	2.33
<i>EPHB4</i>	EPH receptor B4	2.32
<i>GNG4</i>	guanine nucleotide binding protein (G protein), gamma 4	2.32
<i>SLC9A3R1</i>	solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1	2.31
<i>IQGAP2</i>	IQ motif containing GTPase activating protein 2	2.31

Symbol	Gene name	Fold cahnge
<i>SYNCRIP</i>	synaptotagmin binding, cytoplasmic RNA interacting protein	2.31
<i>AKAP7</i>	A kinase (PRKA) anchor protein 7	2.31
<i>F2</i>	coagulation factor II (thrombin)	2.31
<i>PCTP</i>	phosphatidylcholine transfer protein	2.30
<i>FRAT1</i>	frequently rearranged in advanced T-cell lymphomas	2.30
<i>WASF1</i>	WAS protein family, member 1	2.30
<i>SERPINF1</i>	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	2.30
<i>LAPTM4A</i>	lysosomal-associated protein transmembrane 4 alpha	2.30
<i>DMD</i>	dystrophin (muscular dystrophy, Duchenne and Becker types)	2.29
<i>TM9SF1</i>	transmembrane 9 superfamily member 1	2.29
<i>ROBO1</i>	roundabout, axon guidance receptor, homolog 1 (Drosophila)	2.29
<i>MINPP1</i>	multiple inositol polyphosphate histidine phosphatase, 1	2.28
<i>NAB1</i>	NGFI-A binding protein 1 (EGR1 binding protein 1)	2.28
<i>SMARCE1</i>	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1	2.28
<i>PIPOX</i>	pipecolic acid oxidase	2.27
<i>AGL</i>	amyl-1, 6-glucosidase, 4-alpha-glucanotransferase (glycogen debranching enzyme, glycogen storage disease type III)	2.26
<i>PCSK6</i>	proprotein convertase subtilisin/kexin type 6	2.26
<i>ABCB1 /// ABCB4</i>	ATP-binding cassette, sub-family B (MDR/TAP), member 1 /// ATP-binding cassette, sub-family B (MDR/TAP), member 4	2.25
<i>MAGED4</i>	melanoma antigen family D, 4 /// melanoma antigen family D, 4	2.25
<i>CROP</i>	cisplatin resistance-associated overexpressed protein	2.25
<i>CDH2</i>	cadherin 2, type 1, N-cadherin (neuronal)	2.25
<i>LAMB2</i>	laminin, beta 2 (laminin S)	2.24
<i>FAM8A1</i>	family with sequence similarity 8, member A1	2.24
<i>NBR1 /// LOC727732</i>	neighbor of BRCA1 gene 1 /// similar to neighbor of BRCA1 gene 1	2.24
<i>CX3CL1</i>	chemokine (C-X3-C motif) ligand 1	2.24
<i>TF</i>	transferrin	2.24
<i>6-Mar</i>	membrane-associated ring finger (C3HC4) 6	2.23
<i>ARL5A</i>	ADP-ribosylation factor-like 5A	2.23
<i>ACVR1</i>	activin A receptor, type I	2.23
<i>ERBB3</i>	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	2.23
<i>HSD17B7 /// HSD17B7P2 /// LOC730412</i>	hydroxysteroid (17-beta) dehydrogenase 7 /// hydroxysteroid (17-beta) dehydrogenase 7 pseudogene 2 /// similar to hydroxysteroid (17-beta) dehydrogenase 7	2.22
<i>KLF9</i>	Kruppel-like factor 9	2.22
<i>ARID3A</i>	AT rich interactive domain 3A (BRIGHT-like)	2.22
<i>GCH1</i>	GTP cyclohydrolase 1 (dopa-responsive dystonia)	2.22
<i>CPVL</i>	carboxypeptidase, vitellogenic-like /// carboxypeptidase, vitellogenic-like	2.21
<i>HSD17B12</i>	hydroxysteroid (17-beta) dehydrogenase 12	2.21
<i>ZNF124</i>	zinc finger protein 124	2.21
<i>KLHDC2</i>	kelch domain containing 2	2.21
<i>C2</i>	complement component 2	2.21
<i>TANK</i>	TRAF family member-associated NFKB activator	2.21
<i>VPS54</i>	vacuolar protein sorting 54 homolog (S. cerevisiae)	2.21
<i>MUT</i>	methylmalonyl Coenzyme A mutase	2.20
<i>CTNNBIP1</i>	catenin, beta interacting protein 1	2.20

Symbol	Gene name	Fold cahnge
<i>GALNT1</i>	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1 (GalNAc-T1)	2.20
<i>NDRG2</i>	NDRG family member 2	2.19
<i>PLEKHA1</i>	pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 1	2.19
<i>PECR</i>	peroxisomal trans-2-enoyl-CoA reductase	2.19
<i>GNPAT</i>	glyceronephosphate O-acyltransferase	2.19
<i>CYB5A</i>	cytochrome b5 type A (microsomal)	2.19
<i>RNF13</i>	ring finger protein 13	2.19
<i>SMARCA5</i>	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5	2.18
<i>METTL1</i>	methyltransferase like 1	2.18
<i>DDB2</i>	damage-specific DNA binding protein 2, 48kDa	2.18
<i>PAPSS1</i>	3'-phosphoadenosine 5'-phosphosulfate synthase 1	2.17
	CDNA clone IMAGE:5314178 /// CDNA clone IMAGE:5314178	2.17
<i>SLC7A9</i>	solute carrier family 7 (cationic amino acid transporter, y+ system), member 9	2.17
<i>RPL15</i>	ribosomal protein L15	2.16
<i>EPB41L2</i>	erythrocyte membrane protein band 4.1-like 2	2.15
<i>BET1</i>	BET1 homolog (S. cerevisiae)	2.15
<i>GCS1</i>	glucosidase I	2.15
<i>HAGH</i>	hydroxyacylglutathione hydrolase	2.15
<i>LAMP2</i>	lysosomal-associated membrane protein 2	2.14
<i>PRKAR1A</i>	protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1)	2.14
<i>ACVR2B</i>	activin A receptor, type IIB	2.14
<i>F7</i>	coagulation factor VII (serum prothrombin conversion accelerator)	2.13
<i>SPFH2</i>	SPFH domain family, member 2	2.13
<i>GLUL</i>	glutamate-ammonia ligase (glutamine synthetase)	2.13
<i>CXADR</i>	coxsackie virus and adenovirus receptor	2.13
<i>DDC</i>	dopa decarboxylase (aromatic L-amino acid decarboxylase)	2.12
<i>SAA4</i>	serum amyloid A4, constitutive	2.12
<i>TRIM8</i>	tripartite motif-containing 8 /// tripartite motif-containing 8	2.12
<i>PTK7</i>	PTK7 protein tyrosine kinase 7	2.11
<i>PIAS1</i>	protein inhibitor of activated STAT, 1	2.11
<i>GGH</i>	gamma-glutamyl hydrolase (conjugase, folylpolygamma-glutamyl hydrolase)	2.11
<i>GRHPR</i>	glyoxylate reductase/hydroxypyruvate reductase	2.11
<i>15-Sep</i>	15 kDa selenoprotein	2.10
<i>SNRPN</i> /// <i>SNURF</i>	small nuclear ribonucleoprotein polypeptide N /// SNRPN upstream reading frame	2.10
<i>RABEPK</i>	Rab9 effector protein with kelch motifs	2.10
<i>PPAP2B</i>	phosphatidic acid phosphatase type 2B	2.09
<i>H2AFV</i>	H2A histone family, member V	2.09
<i>EGR1</i>	early growth response 1	2.09
<i>BCAM</i>	basal cell adhesion molecule (Lutheran blood group)	2.09
<i>IGFBP3</i>	insulin-like growth factor binding protein 3	2.09
<i>PHYH</i>	phytanoyl-CoA 2-hydroxylase	2.08
<i>SYPL1</i>	synaptophysin-like 1	2.08
<i>ITM2B</i>	integral membrane protein 2B	2.07
<i>PGCP</i>	plasma glutamate carboxypeptidase	2.07
<i>ATP1B1</i>	ATPase, Na+/K+ transporting, beta 1 polypeptide	2.06

Symbol	Gene name	Fold cahnge
<i>CRYL1</i>	crystallin, lambda 1	2.06
<i>MGC5139</i>	hypothetical protein MGC5139	2.06
<i>LAP3</i>	leucine aminopeptidase 3	2.06
<i>CLU</i>	clusterin	2.06
<i>HSD17B4</i>	hydroxysteroid (17-beta) dehydrogenase 4	2.06
<i>AHSG</i>	alpha-2-HS-glycoprotein	2.05
<i>DKK1</i>	dickkopf homolog 1 (Xenopus laevis)	2.05
<i>GPX1</i>	glutathione peroxidase 1	2.04
<i>GMCL1</i>	germ cell-less homolog 1 (Drosophila)	2.04
<i>CETN2</i>	centrin, EF-hand protein, 2	2.04
<i>SPAG4</i>	sperm associated antigen 4	2.04
<i>PPOX</i>	protoporphyrinogen oxidase	2.04
<i>MAN2A1</i>	mannosidase, alpha, class 2A, member 1	2.04
<i>IL1RN</i>	interleukin 1 receptor antagonist	2.03
<i>ZBED5</i>	zinc finger, BED-type containing 5	2.03
<i>RBM8A</i>	RNA binding motif protein 8A	2.03
<i>BFAR</i>	bifunctional apoptosis regulator	2.03
<i>SEC63</i>	SEC63 homolog (S. cerevisiae)	2.03
<i>SNAPC1</i>	small nuclear RNA activating complex, polypeptide 1, 43kDa	2.03
<i>PTPRF</i>	protein tyrosine phosphatase, receptor type, F	2.02
<i>FGG</i>	fibrinogen gamma chain	2.02
<i>WVVOX</i>	WW domain containing oxidoreductase	2.02
<i>SLCO4A1</i>	solute carrier organic anion transporter family, member 4A1	2.02
<i>ATP2B1</i>	ATPase, Ca++ transporting, plasma membrane 1	2.02
<i>FBXO5</i>	F-box protein 5	2.01
<i>CHES1</i>	checkpoint suppressor 1	2.01
<i>PABPN1</i>	poly(A) binding protein, nuclear 1	2.00
<i>BCL6</i>	B-cell CLL/lymphoma 6 (zinc finger protein 51) /// B-cell CLL/lymphoma 6 (zinc finger protein 51)	2.00
<i>RASSF3</i>	Ras association (RalGDS/AF-6) domain family 3	2.00
<i>LANCL1</i>	LanC lantibiotic synthetase component C-like 1 (bacterial)	2.00

5 a) Up-regulated genes in 40 µM exposure

Symbol	Gene name	Fold change
<i>HMOX1</i>	heme oxygenase (decycling) 1	59.00
<i>DUSP5</i>	dual specificity phosphatase 5	46.14
<i>RBP1</i>	retinol binding protein 1, cellular	25.68
<i>GADD45B</i>	growth arrest and DNA-damage-inducible, beta	22.61
<i>ADM</i>	adrenomedullin	19.33
<i>TIMP1</i>	TIMP metalloproteinase inhibitor 1	18.90
<i>CAPN2</i>	calpain 2, (mII) large subunit	18.27
<i>HSPA1A</i> /// <i>HSPA1B</i>	heat shock 70kDa protein 1A /// heat shock 70kDa protein 1B	17.32
<i>EMP3</i>	epithelial membrane protein 3	16.33
<i>PHLDA2</i>	pleckstrin homology-like domain, family A, member 2	16.17
<i>FHL2</i>	four and a half LIM domains 2	16.04
<i>MT1X</i>	metallothionein 1X	13.58
<i>PPP1R15A</i>	protein phosphatase 1, regulatory (inhibitor) subunit 15A	12.04
<i>AQP3</i>	aquaporin 3 (Gill blood group)	11.65
<i>GCNT3</i>	glucosaminyl (N-acetyl) transferase 3, mucin type	11.64
<i>UPP1</i>	uridine phosphorylase 1	11.28
<i>TAGLN</i>	transgelin	10.91
<i>HMGA1</i>	high mobility group AT-hook 1	10.73
<i>S100A6</i>	S100 calcium binding protein A6	10.25
<i>CDKN1A</i>	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	10.19
<i>KRT19</i>	keratin 19	9.85
<i>CALB2</i>	calbindin 2, 29kDa (calretinin)	9.34
<i>CYR61</i>	cysteine-rich, angiogenic inducer, 61	9.32
<i>RIT1</i>	Ras-like without CAAX 1	8.98
<i>FOSL1</i>	FOS-like antigen 1	8.93
<i>TOMM34</i>	translocase of outer mitochondrial membrane 34	8.60
<i>NP</i>	nucleoside phosphorylase	8.58
<i>GCLM</i>	glutamate-cysteine ligase, modifier subunit	8.34
<i>SFN</i>	stratifin	8.20
<i>TPM4</i>	tropomyosin 4	7.84
<i>ATP6V1D</i>	ATPase, H+ transporting, lysosomal 34kDa, V1 subunit D	7.73
<i>UCHL3</i>	ubiquitin carboxyl-terminal esterase L3 (ubiquitin thioesterase)	7.63
<i>PITPNC1</i>	phosphatidylinositol transfer protein, cytoplasmic 1	7.56
<i>BLVRB</i>	biliverdin reductase B (flavin reductase (NADPH))	7.50
<i>GRB10</i>	growth factor receptor-bound protein 10	7.33
<i>HSPA1B</i>	heat shock 70kDa protein 1B	7.32
<i>RHOC</i>	ras homolog gene family, member C	7.31
<i>PLK2</i>	polo-like kinase 2 (Drosophila)	7.07
<i>PEA15</i>	phosphoprotein enriched in astrocytes 15	6.97
<i>BHMT2</i>	betaine-homocysteine methyltransferase 2	6.96
<i>HIST1H1C</i>	histone cluster 1, H1c	6.84
<i>ATP6V1C1</i>	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1	6.77
<i>LGALS3</i>	lectin, galactoside-binding, soluble, 3 (galectin 3)	6.65
<i>F2RL1</i>	coagulation factor II (thrombin) receptor-like 1	6.65
<i>ANXA3</i>	annexin A3	6.56
<i>MT1X</i>	metallothionein 1X	6.51

Symbol	Gene name	Fold change
<i>MVP</i>	major vault protein	6.51
<i>RAI14</i>	retinoic acid induced 14	6.45
<i>CYB5R4</i>	cytochrome b5 reductase 4	6.43
<i>MAFF</i>	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	6.24
<i>TUBB3</i>	tubulin, beta 3	6.17
<i>VNN1</i>	vanin 1 /// vanin 1	6.12
<i>CD55</i>	CD55 molecule, decay accelerating factor for complement (Cromer blood group)	6.07
<i>SFN</i>	stratifin	5.95
<i>MT1H</i>	metallothionein 1H	5.86
<i>RAB5C</i>	RAB5C, member RAS oncogene family	5.77
<i>MSX1</i>	msh homeobox 1	5.76
<i>MYLK</i>	myosin, light chain kinase /// myosin, light chain kinase	5.75
<i>CTSL</i>	cathepsin L	5.74
<i>RAB27A</i>	RAB27A, member RAS oncogene family	5.70
<i>SEC14L1</i>	SEC14-like 1 (S. cerevisiae)	5.68
<i>ZYX</i>	zyxin	5.66
<i>CTSA</i>	cathepsin A	5.61
<i>GBE1</i>	glucan (1,4-alpha-), branching enzyme 1 (glycogen branching enzyme, Andersen disease, glycogen storage disease type IV)	5.55
<i>HPCAL1</i>	hippocalcin-like 1	5.54
<i>MT2A</i>	metallothionein 2A	5.52
<i>TGFB1</i>	transforming growth factor, beta 1 (Camurati-Engelmann disease)	5.46
<i>ALAS1</i>	aminolevulinate, delta-, synthase 1	5.39
<i>PSMD2</i>	proteasome (prosome, macropain) 26S subunit, non-ATPase, 2	5.32
<i>RHOQ</i>	ras homolog gene family, member Q	5.28
<i>HSPH1</i>	heat shock 105kDa/110kDa protein 1	5.21
<i>TLE3</i>	transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)	5.16
<i>GLRX</i>	glutaredoxin (thioltransferase)	5.16
<i>KYNU</i>	kynureninase (L-kynurenine hydrolase)	5.15
<i>LGALS1</i>	lectin, galactoside-binding, soluble, 1 (galectin 1)	5.11
<i>TRPV2</i>	transient receptor potential cation channel, subfamily V, member 2	5.11
<i>GSK3B</i>	glycogen synthase kinase 3 beta	5.07
<i>SVIL</i>	supervillin	5.01
<i>TINF2</i>	TERF1 (TRF1)-interacting nuclear factor 2	4.95
<i>SQRDL</i>	sulfide quinone reductase-like (yeast)	4.95
<i>M6PRBP1</i>	mannose-6-phosphate receptor binding protein 1	4.94
<i>AKR1B1</i>	aldo-keto reductase family 1, member B1 (aldose reductase)	4.92
<i>EHD1</i>	EH-domain containing 1	4.91
<i>BPGM</i>	2,3-bisphosphoglycerate mutase /// 2,3-bisphosphoglycerate mutase	4.90
<i>CALM1</i>	calmodulin 1 (phosphorylase kinase, delta)	4.86
<i>VAT1</i>	vesicle amine transport protein 1 homolog (T. californica)	4.86
<i>SIRT7</i> /// <i>LOC644124</i>	sirtuin (silent mating type information regulation 2 homolog) 7 (S. cerevisiae) /// similar to NAD-dependent deacetylase sirtuin-7 (SIR2-like protein 7)	4.86
<i>SNN</i>	stannin	4.80
<i>UBL3</i>	ubiquitin-like 3	4.78
<i>PMP22</i>	peripheral myelin protein 22	4.78
<i>DTNA</i>	dystrobrevin, alpha	4.77
<i>BNIP3L</i>	BCL2/adenovirus E1B 19kDa interacting protein 3-like /// BCL2/adenovirus E1B 19kDa interacting protein 3-like	4.77

Symbol	Gene name	Fold change
<i>HIST1H2BK</i>	histone cluster 1, H2bk	4.73
<i>ASPH</i>	aspartate beta-hydroxylase	4.73
<i>TRIM38</i>	tripartite motif-containing 38	4.72
<i>RAB22A</i>	RAB22A, member RAS oncogene family	4.65
<i>CD58</i>	CD58 molecule	4.62
<i>SH3BP4</i>	SH3-domain binding protein 4	4.61
<i>MKLN1</i>	muskelin 1, intracellular mediator containing kelch motifs	4.61
<i>UBE2H</i>	ubiquitin-conjugating enzyme E2H (UBC8 homolog, yeast)	4.54
<i>TSPAN13</i>	Tetraspanin 13	4.53
<i>S100A10</i>	S100 calcium binding protein A10	4.51
<i>ZNF267</i>	zinc finger protein 267	4.51
<i>SLC2A3</i>	solute carrier family 2 (facilitated glucose transporter), member 3	4.50
<i>GNPDA1</i>	glucosamine-6-phosphate deaminase 1	4.50
<i>MAP2K1</i>	mitogen-activated protein kinase kinase 1	4.46
<i>HIST1H2BK</i>	histone cluster 1, H2bk	4.42
<i>HSPB1</i> /// <i>MEIS3</i>	heat shock 27kDa protein 1 /// Meis1, myeloid ecotropic viral integration site 1 homolog 3 (mouse)	4.41
<i>KPNA4</i>	karyopherin alpha 4 (importin alpha 3)	4.40
<i>SDCBP</i>	syndecan binding protein (syntenin)	4.40
<i>STX3</i>	syntaxin 3	4.33
	CDNA FLJ34214 fis, clone FCBBF3021807	4.32
<i>FLNA</i>	filamin A, alpha (actin binding protein 280)	4.23
<i>GDI1</i>	GDP dissociation inhibitor 1	4.22
<i>PSMD3</i>	proteasome (prosome, macropain) 26S subunit, non-ATPase, 3	4.20
<i>COTL1</i>	coactosin-like 1 (Dictyostelium)	4.20
<i>HOMER3</i>	homer homolog 3 (Drosophila)	4.16
<i>IRS2</i>	insulin receptor substrate 2	4.12
<i>ADRM1</i>	adhesion regulating molecule 1	4.11
<i>CDC37</i>	cell division cycle 37 homolog (S. cerevisiae)	4.07
<i>BAG3</i>	BCL2-associated athanogene 3	4.02
<i>CAP1</i>	CAP, adenylate cyclase-associated protein 1 (yeast)	3.97
<i>TXNRD1</i>	thioredoxin reductase 1	3.95
<i>VIL2</i>	villin 2 (ezrin)	3.94
<i>SHC1</i>	SHC (Src homology 2 domain containing) transforming protein 1	3.92
<i>MAP2K2</i>	mitogen-activated protein kinase kinase 2	3.90
<i>PNMA1</i>	paraneoplastic antigen MA1	3.87
<i>NDRG1</i>	N-myc downstream regulated gene 1	3.87
<i>IQGAP1</i>	IQ motif containing GTPase activating protein 1	3.84
<i>BTG3</i>	BTG family, member 3	3.83
<i>ZNF238</i>	zinc finger protein 238	3.83
<i>DRAP1</i>	DR1-associated protein 1 (negative cofactor 2 alpha)	3.81
<i>MAP2K1IP1</i>	mitogen-activated protein kinase kinase 1 interacting protein 1	3.81
<i>ATP6V1E1</i>	ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E1	3.79
<i>DNAJB4</i>	DnaJ (Hsp40) homolog, subfamily B, member 4	3.78
<i>TTG4</i> /// <i>C1orf175</i>	tetratricopeptide repeat domain 4 /// chromosome 1 open reading frame 175	3.76
<i>C1orf41</i> /// <i>IL17RB</i>	chromosome 1 open reading frame 41 /// interleukin 17 receptor B	3.73
<i>ATG7</i>	ATG7 autophagy related 7 homolog (S. cerevisiae)	3.73
<i>CCNE2</i>	cyclin E2	3.72

Symbol	Gene name	Fold change
<i>ATF5</i>	activating transcription factor 5	3.72
<i>NAGK</i>	N-acetylglucosamine kinase /// N-acetylglucosamine kinase	3.71
<i>GLA</i>	galactosidase, alpha	3.69
<i>RIOK3</i>	RIO kinase 3 (yeast) /// RIO kinase 3 (yeast)	3.69
<i>TUBB2C</i>	tubulin, beta 2C	3.67
<i>UBE2S</i> /// <i>LOC731049</i>	ubiquitin-conjugating enzyme E2S /// similar to Ubiquitin-conjugating enzyme E2S (Ubiquitin-conjugating enzyme E2-24 kDa) (Ubiquitin-protein ligase) (Ubiquitin carrier protein) (E2-EPF5)	3.66
<i>PCQAP</i>	PC2 (positive cofactor 2, multiprotein complex) glutamine/Q-rich-associated protein	3.63
<i>ABCC1</i>	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	3.63
<i>TMEM111</i>	transmembrane protein 111	3.63
<i>CD24</i>	CD24 molecule	3.60
<i>CES1</i>	carboxylesterase 1 (monocyte/macrophage serine esterase 1)	3.59
<i>GYS1</i>	glycogen synthase 1 (muscle)	3.58
<i>SKAP2</i>	src kinase associated phosphoprotein 2	3.56
<i>MAP1LC3B</i>	microtubule-associated protein 1 light chain 3 beta	3.56
<i>ARPC5L</i>	actin related protein 2/3 complex, subunit 5-like /// actin related protein 2/3 complex, subunit 5-like	3.56
<i>PLAA</i>	phospholipase A2-activating protein	3.55
<i>BCAR3</i>	breast cancer anti-estrogen resistance 3	3.54
<i>FLII</i>	flightless I homolog (Drosophila)	3.52
<i>NMB</i>	neuromedin B	3.52
<i>AKAP12</i>	A kinase (PRKA) anchor protein (gravin) 12	3.50
<i>PTPN3</i>	protein tyrosine phosphatase, non-receptor type 3	3.49
<i>HIST2H2AA3</i> /// <i>HIST2H2AA4</i>	histone cluster 2, H2aa3 /// histone cluster 2, H2aa4	3.47
<i>ATF3</i>	activating transcription factor 3	3.47
<i>DUSP10</i>	dual specificity phosphatase 10	3.45
<i>STAM</i>	signal transducing adaptor molecule (SH3 domain and ITAM motif) 1	3.44
<i>TRIP12</i>	thyroid hormone receptor interactor 12	3.44
<i>TPD52</i>	tumor protein D52	3.43
<i>AFF1</i>	AF4/FMR2 family, member 1	3.42
<i>CD2BP2</i>	CD2 (cytoplasmic tail) binding protein 2	3.41
<i>TALDO1</i>	transaldolase 1	3.40
<i>DUSP1</i>	dual specificity phosphatase 1	3.39
<i>LOC729659</i> /// <i>LOC730278</i> /// <i>LOC730558</i>	similar to Putative S100 calcium-binding protein A11 pseudogene /// similar to Putative S100 calcium-binding protein A11 pseudogene /// similar to Putative S100 calcium-binding protein A11 pseudogene	3.39
<i>SERPINE1</i>	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	3.38
<i>RNF14</i>	ring finger protein 14	3.38
<i>LYPLA3</i>	lysophospholipase 3 (lysosomal phospholipase A2)	3.36
<i>DDX24</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 24	3.35
<i>WSB2</i>	WD repeat and SOCS box-containing 2	3.33
<i>ATP6V0C</i>	ATPase, H+ transporting, lysosomal 16kDa, V0 subunit c	3.32
<i>UBE2E3</i>	ubiquitin-conjugating enzyme E2E 3 (UBC4/5 homolog, yeast)	3.32
<i>PSMD13</i>	proteasome (prosome, macropain) 26S subunit, non-ATPase, 13	3.31
<i>NAPG</i>	N-ethylmaleimide-sensitive factor attachment protein, gamma	3.31
<i>GSTM3</i>	glutathione S-transferase M3 (brain)	3.30
<i>KNS2</i>	kinesin 2	3.29

Symbol	Gene name	Fold change
<i>DSTN</i>	destrin (actin depolymerizing factor)	3.29
<i>UBQLN2</i>	ubiquilin 2	3.28
<i>C12orf4</i>	chromosome 12 open reading frame 4	3.28
<i>LMNA</i>	lamin A/C	3.28
<i>ACTN1</i>	actinin, alpha 1	3.27
<i>PSMD14</i>	proteasome (prosome, macropain) 26S subunit, non-ATPase, 14	3.27
<i>ATP6V1F</i>	ATPase, H+ transporting, lysosomal 14kDa, V1 subunit F	3.26
<i>RRAGC</i>	Ras-related GTP binding C	3.26
<i>PKM2</i>	pyruvate kinase, muscle	3.23
<i>RTN2</i>	reticulon 2	3.22
<i>TUFT1</i>	tuftelin 1	3.22
<i>C20orf24</i>	chromosome 20 open reading frame 24	3.21
<i>YKT6</i>	YKT6 v-SNARE homolog (S. cerevisiae)	3.20
<i>DNAJB1</i>	DnaJ (Hsp40) homolog, subfamily B, member 1	3.20
<i>VCP</i>	valosin-containing protein	3.19
<i>LASP1</i>	LIM and SH3 protein 1	3.19
<i>DDEF2</i>	development and differentiation enhancing factor 2	3.18
<i>NRD1</i>	nardilysin (N-arginine dibasic convertase)	3.18
<i>SEC23B</i>	Sec23 homolog B (S. cerevisiae)	3.18
<i>CTNNAL1</i>	catenin (cadherin-associated protein), alpha-like 1	3.15
<i>BHLHB2</i>	basic helix-loop-helix domain containing, class B, 2	3.15
<i>MOAP1</i>	modulator of apoptosis 1	3.15
<i>RAB11A</i>	RAB11A, member RAS oncogene family	3.15
<i>HIST2H2BE</i>	histone cluster 2, H2be	3.13
<i>SP110</i>	SP110 nuclear body protein	3.12
<i>MYO6</i>	myosin VI	3.11
<i>RALA</i>	v-ral simian leukemia viral oncogene homolog A (ras related)	3.08
<i>SMURF2</i>	SMAD specific E3 ubiquitin protein ligase 2	3.08
<i>HGS</i>	hepatocyte growth factor-regulated tyrosine kinase substrate	3.07
<i>POLR2C</i>	polymerase (RNA) II (DNA directed) polypeptide C, 33kDa	3.06
<i>PSMC4 /// LOC652826</i>	proteasome (prosome, macropain) 26S subunit, ATPase, 4 /// similar to 26S protease regulatory subunit 6B (MIP224) (MB67-interacting protein) (TAT-binding protein 7) (TBP-7)	3.06
<i>ASNA1</i>	arsA arsenite transporter, ATP-binding, homolog 1 (bacterial)	3.05
<i>RASSF2</i>	Ras association (RaGDS/AF-6) domain family 2	3.04
<i>ALDH9A1</i>	aldehyde dehydrogenase 9 family, member A1	3.04
<i>MAPK13</i>	mitogen-activated protein kinase 13	3.03
<i>HPS5</i>	Hermansky-Pudlak syndrome 5	3.02
<i>AVEN</i>	apoptosis, caspase activation inhibitor	3.01
<i>CACYBP</i>	calcyclin binding protein	3.01
<i>ECD</i>	ecdysoneless homolog (Drosophila)	3.00
<i>PSMB2</i>	proteasome (prosome, macropain) subunit, beta type, 2 /// proteasome (prosome, macropain) subunit, beta type, 2	2.99
<i>HIVEP2</i>	human immunodeficiency virus type I enhancer binding protein 2	2.98
<i>CD63</i>	CD63 molecule	2.98
<i>AP2S1</i>	adaptor-related protein complex 2, sigma 1 subunit	2.97
<i>TNFRSF12A</i>	tumor necrosis factor receptor superfamily, member 12A	2.97
<i>SQSTM1</i>	sequestosome 1	2.95
<i>PSMD8</i>	proteasome (prosome, macropain) 26S subunit, non-ATPase, 8	2.94

Symbol	Gene name	Fold change
<i>SNX11</i>	sorting nexin 11	2.94
	CDNA clone IMAGE:3030163	2.94
<i>NRBP1</i>	nuclear receptor binding protein 1	2.92
<i>TNFRSF10D</i>	tumor necrosis factor receptor superfamily, member 10d, decoy with truncated death domain	2.92
<i>SF3B4</i>	splicing factor 3b, subunit 4, 49kDa	2.92
<i>OAS1</i>	2',5'-oligoadenylate synthetase 1, 40/46kDa	2.92
<i>ACOT8</i>	acyl-CoA thioesterase 8	2.91
<i>TMEM8</i>	transmembrane protein 8 (five membrane-spanning domains)	2.90
<i>HLA3</i>	HERV-H LTR-associating 3	2.90
<i>PSMD12</i>	proteasome (prosome, macropain) 26S subunit, non-ATPase, 12	2.89
<i>PPP2CB</i>	protein phosphatase 2 (formerly 2A), catalytic subunit, beta isoform	2.89
<i>BAZ1A</i>	bromodomain adjacent to zinc finger domain, 1A	2.88
<i>CHIC2</i>	cysteine-rich hydrophobic domain 2	2.87
<i>SPAG1</i>	sperm associated antigen 1	2.87
<i>STK17A</i>	serine/threonine kinase 17a (apoptosis-inducing)	2.87
<i>EPHX1</i>	epoxide hydrolase 1, microsomal (xenobiotic)	2.86
<i>YWHAZ</i>	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	2.86
<i>MEA1</i>	male-enhanced antigen 1	2.85
<i>CLPTM1</i>	cleft lip and palate associated transmembrane protein 1	2.85
<i>HEY1</i>	hairy/enhancer-of-split related with YRPW motif 1	2.84
<i>GADD45A</i>	growth arrest and DNA-damage-inducible, alpha	2.84
<i>NSFL1C</i>	NSFL1 (p97) cofactor (p47)	2.83
<i>NDUFB3</i>	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa	2.83
<i>TCEB1</i>	transcription elongation factor B (SIII), polypeptide 1 (15kDa, elongin C)	2.83
<i>CAP2</i>	CAP, adenylate cyclase-associated protein, 2 (yeast)	2.83
<i>SERPINE2</i>	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2	2.82
<i>SYBL1</i>	synaptobrevin-like 1	2.81
<i>PANX1</i>	pannexin 1	2.81
<i>HIST1H2AC</i>	histone cluster 1, H2ac	2.80
<i>LRP8</i>	low density lipoprotein receptor-related protein 8, apolipoprotein e receptor	2.80
<i>ATP6V1B2</i>	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B2	2.80
<i>NAPA</i>	N-ethylmaleimide-sensitive factor attachment protein, alpha	2.79
<i>FKBP1A</i>	FK506 binding protein 1A, 12kDa	2.78
<i>RTCD1</i>	RNA terminal phosphate cyclase domain 1	2.78
<i>ARPC4</i>	actin related protein 2/3 complex, subunit 4, 20kDa	2.78
<i>MTMR2</i>	myotubularin related protein 2	2.78
<i>GIPC1</i>	GIPC PDZ domain containing family, member 1	2.77
<i>UFD1L</i>	ubiquitin fusion degradation 1 like (yeast)	2.77
<i>PPP1R11</i>	protein phosphatase 1, regulatory (inhibitor) subunit 11	2.77
<i>PITPNA</i>	phosphatidylinositol transfer protein, alpha	2.76
<i>VRK3</i>	vaccinia related kinase 3	2.76
<i>TAX1BP1</i>	Tax1 (human T-cell leukemia virus type I) binding protein 1	2.76
<i>ING1</i>	inhibitor of growth family, member 1	2.75
<i>RORA</i>	RAR-related orphan receptor A	2.75
<i>ARFGEF1</i>	ADP-ribosylation factor guanine nucleotide-exchange factor 1(brefeldin A-inhibited)	2.75
<i>RTN4</i>	reticulon 4	2.74

Symbol	Gene name	Fold change
<i>WBP5</i>	WW domain binding protein 5	2.74
<i>LRRFIP1</i>	leucine rich repeat (in FLII) interacting protein 1	2.74
<i>RAP1GDS1</i>	RAP1, GTP-GDP dissociation stimulator 1	2.74
<i>HIST1H2BD</i>	histone cluster 1, H2bd	2.72
<i>BLCAP</i>	bladder cancer associated protein	2.71
<i>HEBP1</i>	heme binding protein 1	2.70
<i>MRCL3 /// MRLC2</i>	myosin regulatory light chain MRCL3 /// myosin regulatory light chain MRLC2	2.70
<i>ATP6V0E1</i>	ATPase, H+ transporting, lysosomal 9kDa, V0 subunit e1 /// ATPase, H+ transporting, lysosomal 9kDa, V0 subunit e1	2.69
<i>SP110</i>	SP110 nuclear body protein	2.69
<i>AATF</i>	apoptosis antagonizing transcription factor	2.69
<i>TNIP1</i>	TNFAIP3 interacting protein 1	2.69
<i>POLR3C</i>	polymerase (RNA) III (DNA directed) polypeptide C (62kD)	2.68
<i>PCOLN3</i>	procollagen (type III) N-endopeptidase	2.68
<i>CBX4</i>	chromobox homolog 4 (Pc class homolog, Drosophila)	2.67
<i>PPP2R2A</i>	protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), alpha isoform	2.67
<i>PRAME</i>	preferentially expressed antigen in melanoma	2.66
<i>CFL1</i>	cofilin 1 (non-muscle) /// cofilin 1 (non-muscle)	2.66
<i>PRR13</i>	proline rich 13	2.65
<i>ELOVL1</i>	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 1	2.64
<i>MKNK2</i>	MAP kinase interacting serine/threonine kinase 2	2.64
<i>ACTR1A</i>	ARP1 actin-related protein 1 homolog A, centractin alpha (yeast)	2.64
<i>APEX2</i>	APEX nuclease (apurinic/apyrimidinic endonuclease) 2	2.64
<i>ING2</i>	inhibitor of growth family, member 2	2.64
<i>CHORDC1</i>	cysteine and histidine-rich domain (CHORD)-containing 1	2.63
<i>RHOG</i>	ras homolog gene family, member G (rho G)	2.62
<i>DIDO1</i>	death inducer-obliterator 1	2.61
<i>AK1</i>	adenylate kinase 1	2.61
<i>PIR</i>	pirin (iron-binding nuclear protein)	2.61
<i>RNF6</i>	ring finger protein (C3H2C3 type) 6	2.61
<i>ZFP36L1</i>	zinc finger protein 36, C3H type-like 1	2.60
<i>CREB3</i>	cAMP responsive element binding protein 3	2.60
<i>C18orf8</i>	chromosome 18 open reading frame 8	2.59
<i>C1S</i>	complement component 1, s subcomponent	2.59
<i>KCMF1</i>	potassium channel modulatory factor 1	2.59
<i>ARID1A</i>	AT rich interactive domain 1A (SWI-like)	2.59
<i>HEBP2</i>	heme binding protein 2	2.58
<i>ARF3</i>	ADP-ribosylation factor 3 /// ADP-ribosylation factor 3	2.58
<i>ATG3</i>	ATG3 autophagy related 3 homolog (S. cerevisiae)	2.58
<i>MRLC2</i>	myosin regulatory light chain MRLC2	2.58
<i>EGR1</i>	early growth response 1	2.58
<i>ZFAND6</i>	zinc finger, AN1-type domain 6	2.57
<i>SPAG9</i>	sperm associated antigen 9	2.57
<i>TMC6</i>	transmembrane channel-like 6	2.56
<i>SGTA</i>	small glutamine-rich tetratricopeptide repeat (TPR)-containing, alpha	2.56
<i>PFN1</i>	profilin 1	2.55
<i>LSM1</i>	LSM1 homolog, U6 small nuclear RNA associated (S. cerevisiae)	2.55
<i>CORO1C</i>	coronin, actin binding protein, 1C	2.55

Symbol	Gene name	Fold change
<i>FAM53C</i>	family with sequence similarity 53, member C	2.55
<i>TRIP4</i>	thyroid hormone receptor interactor 4	2.55
<i>NR1H2</i>	nuclear receptor subfamily 1, group H, member 2	2.55
<i>FAS</i>	Fas (TNF receptor superfamily, member 6)	2.55
<i>ACVR1</i>	activin A receptor, type I	2.54
<i>STIP1</i>	stress-induced-phosphoprotein 1 (Hsp70/Hsp90-organizing protein)	2.53
<i>PPP3CA</i>	protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform (calcineurin A alpha)	2.53
<i>ATP6V0A1</i>	ATPase, H+ transporting, lysosomal V0 subunit a1	2.53
<i>MARK3</i>	MAP/microtubule affinity-regulating kinase 3	2.52
<i>MYH9</i>	myosin, heavy chain 9, non-muscle	2.52
<i>TRAFD1</i>	TRAF-type zinc finger domain containing 1	2.51
<i>CD9</i>	CD9 molecule	2.50
<i>FBXO2</i>	F-box protein 2	2.50
<i>UBE2M</i>	ubiquitin-conjugating enzyme E2M (UBC12 homolog, yeast)	2.49
<i>NUDC</i>	nuclear distribution gene C homolog (A. nidulans)	2.48
<i>NCKAP1</i>	NCK-associated protein 1	2.48
<i>PARP2</i>	poly (ADP-ribose) polymerase family, member 2	2.48
<i>CMAS</i>	cytidine monophosphate N-acetylneuraminic acid synthetase	2.48
<i>MAPKAPK2</i>	mitogen-activated protein kinase-activated protein kinase 2	2.48
<i>SMS</i>	spermine synthase	2.48
<i>DFNA5</i>	deafness, autosomal dominant 5	2.48
<i>AZIN1</i>	antizyme inhibitor 1	2.48
<i>GRB2</i>	growth factor receptor-bound protein 2	2.48
<i>CALM1</i>	calmodulin 1 (phosphorylase kinase, delta)	2.47
<i>TRIM16 /// TRIM16L</i>	tripartite motif-containing 16 /// tripartite motif-containing 16-like	2.47
<i>C2orf24</i>	chromosome 2 open reading frame 24 /// chromosome 2 open reading frame 24	2.47
<i>CASP7</i>	caspase 7, apoptosis-related cysteine peptidase	2.47
<i>GNB2</i>	guanine nucleotide binding protein (G protein), beta polypeptide 2	2.46
<i>PRIM2A</i>	primase, polypeptide 2A, 58kDa	2.45
<i>PTS</i>	6-pyruvoyltetrahydropterin synthase	2.45
<i>ANXA5</i>	annexin A5	2.45
<i>GRK5</i>	G protein-coupled receptor kinase 5	2.44
<i>VLDLR</i>	very low density lipoprotein receptor	2.44
<i>SMN1 /// SMN2</i>	survival of motor neuron 1, telomeric /// survival of motor neuron 2, centromeric	2.44
<i>RPA2</i>	replication protein A2, 32kDa	2.44
<i>KPNA6</i>	karyopherin alpha 6 (importin alpha 7)	2.44
<i>EXOC5</i>	exocyst complex component 5	2.43
<i>FARS2</i>	phenylalanine-tRNA synthetase 2 (mitochondrial)	2.43
<i>ACTR3</i>	ARP3 actin-related protein 3 homolog (yeast)	2.43
<i>ABHD2</i>	abhydrolase domain containing 2	2.42
<i>GNB5</i>	guanine nucleotide binding protein (G protein), beta 5	2.42
<i>NSF</i>	N-ethylmaleimide-sensitive factor	2.42
<i>SAP30BP</i>	SAP30 binding protein	2.42
<i>SERPINH1</i>	serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)	2.42
<i>PPP1R12A</i>	protein phosphatase 1, regulatory (inhibitor) subunit 12A	2.42
<i>RNF141</i>	ring finger protein 141	2.41

Symbol	Gene name	Fold change
<i>SAR1B</i>	SAR1 gene homolog B (S. cerevisiae)	2.41
<i>ARL6IP5</i>	ADP-ribosylation-like factor 6 interacting protein 5	2.40
<i>NKX3-1</i>	NK3 transcription factor related, locus 1 (Drosophila)	2.40
<i>TRIM23</i>	tripartite motif-containing 23	2.40
<i>ERCC1</i>	excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence)	2.39
<i>PPME1</i>	protein phosphatase methylesterase 1	2.39
<i>SPTAN1</i>	spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	2.39
<i>TSPAN7</i>	tetraspanin 7	2.39
<i>HN1</i>	hematological and neurological expressed 1	2.39
<i>CASP4</i>	caspase 4, apoptosis-related cysteine peptidase	2.38
<i>RAPGEF2</i>	Rap guanine nucleotide exchange factor (GEF) 2	2.38
<i>CSTB</i>	cystatin B (stefin B)	2.38
<i>PRPF18</i>	PRP18 pre-mRNA processing factor 18 homolog (S. cerevisiae)	2.37
<i>PAM</i>	peptidylglycine alpha-amidating monooxygenase	2.37
<i>JAK1</i>	Janus kinase 1 (a protein tyrosine kinase)	2.37
<i>MAFG</i> /// <i>LOC644132</i>	v-maf musculoaponeurotic fibrosarcoma oncogene homolog G (avian) /// similar to v-maf musculoaponeurotic fibrosarcoma oncogene family, protein G	2.37
<i>STRN3</i>	striatin, calmodulin binding protein 3	2.36
<i>PPIF</i>	peptidylprolyl isomerase F (cyclophilin F)	2.36
<i>MSL3L1</i>	male-specific lethal 3-like 1 (Drosophila)	2.35
<i>HAT1</i>	histone acetyltransferase 1	2.35
<i>ITM2C</i>	integral membrane protein 2C /// integral membrane protein 2C	2.35
<i>GTF2B</i>	general transcription factor IIB /// general transcription factor IIB	2.35
<i>DAB2</i>	disabled homolog 2, mitogen-responsive phosphoprotein (Drosophila)	2.35
<i>KLHL2</i>	kelch-like 2, Mayven (Drosophila)	2.34
<i>TXN</i>	thioredoxin	2.34
<i>ACOT7</i>	acyl-CoA thioesterase 7	2.33
<i>DNTTIP2</i>	deoxynucleotidyltransferase, terminal, interacting protein 2	2.33
<i>PSCD2</i>	pleckstrin homology, Sec7 and coiled-coil domains 2 (cytohesin-2)	2.33
<i>MDK</i>	midkine (neurite growth-promoting factor 2)	2.33
<i>NXF1</i>	nuclear RNA export factor 1	2.33
<i>ARG2</i>	arginase, type II	2.32
<i>VCL</i>	vinculin	2.32
<i>CAB39</i>	calcium binding protein 39	2.32
<i>VASP</i>	vasodilator-stimulated phosphoprotein	2.32
<i>SLC12A6</i>	solute carrier family 12 (potassium/chloride transporters), member 6	2.31
<i>AHSA1</i>	AHA1, activator of heat shock 90kDa protein ATPase homolog 1 (yeast)	2.31
<i>C1orf9</i>	chromosome 1 open reading frame 9	2.31
<i>CYB5R1</i>	cytochrome b5 reductase 1	2.30
<i>SLC20A1</i>	solute carrier family 20 (phosphate transporter), member 1	2.30
<i>SKP1A</i>	S-phase kinase-associated protein 1A (p19A)	2.30
<i>PPFIA1</i>	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 1	2.30
<i>RBPM5</i>	RNA binding protein with multiple splicing	2.30
<i>SNX3</i>	sorting nexin 3 /// sorting nexin 3	2.30
<i>NMT1</i>	N-myristoyltransferase 1	2.30
<i>DOCK1</i>	dedicator of cytokinesis 1	2.29
<i>PTPRM</i>	protein tyrosine phosphatase, receptor type, M	2.29

Symbol	Gene name	Fold change
<i>MPP5</i>	membrane protein, palmitoylated 5 (MAGUK p55 subfamily member 5)	2.29
<i>PSMB7</i>	proteasome (prosome, macropain) subunit, beta type, 7	2.29
<i>TNPO3</i>	transportin 3	2.28
<i>ATG12</i>	ATG12 autophagy related 12 homolog (S. cerevisiae)	2.28
<i>DDX39</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39	2.27
<i>STK38</i>	serine/threonine kinase 38	2.27
<i>PNKP</i>	polynucleotide kinase 3'-phosphatase	2.26
<i>ZNF259</i> /// <i>LOC442240</i>	zinc finger protein 259 /// zinc finger protein 259 /// similar to zinc finger protein 259 /// similar to zinc finger protein 259	2.26
<i>CNOT4</i>	CCR4-NOT transcription complex, subunit 4	2.26
<i>EIF1AY</i>	eukaryotic translation initiation factor 1A, Y-linked	2.26
<i>PDCD6IP</i>	programmed cell death 6 interacting protein	2.26
<i>STX4</i>	syntaxin 4	2.25
<i>ISGF3G</i>	interferon-stimulated transcription factor 3, gamma 48kDa	2.25
<i>NDUFA1</i>	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 1, 7.5kDa	2.25
<i>PTP4A2</i>	protein tyrosine phosphatase type IVA, member 2	2.25
<i>HRB</i>	HIV-1 Rev binding protein	2.25
<i>PRKCI</i>	protein kinase C, iota	2.25
<i>GRN</i>	granulin	2.25
<i>MXRA7</i>	matrix-remodelling associated 7	2.24
<i>TRIM32</i>	tripartite motif-containing 32	2.24
<i>ARL4A</i>	ADP-ribosylation factor-like 4A	2.24
<i>BRAF</i>	v-raf murine sarcoma viral oncogene homolog B1	2.24
<i>YWHAH</i>	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide	2.24
<i>PRKACB</i>	protein kinase, cAMP-dependent, catalytic, beta	2.23
<i>CUL3</i>	cullin 3	2.23
<i>CAPNS1</i>	calpain, small subunit 1 /// calpain, small subunit 1	2.23
<i>RRN3</i> /// <i>LOC653390</i> /// <i>LOC730092</i>	RRN3 RNA polymerase I transcription factor homolog (S. cerevisiae) /// hypothetical LOC653390 /// hypothetical protein LOC730092	2.23
<i>TWF1</i>	twinfilin, actin-binding protein, homolog 1 (Drosophila)	2.22
<i>CREM</i>	cAMP responsive element modulator	2.22
<i>VPS16</i>	vacuolar protein sorting 16 homolog (S. cerevisiae)	2.22
<i>GPX3</i>	glutathione peroxidase 3 (plasma)	2.21
<i>TSC1</i>	tuberous sclerosis 1	2.21
<i>ZNF313</i>	zinc finger protein 313	2.21
<i>SLC29A1</i>	solute carrier family 29 (nucleoside transporters), member 1	2.21
<i>RALB</i>	v-rat simian leukemia viral oncogene homolog B (ras related; GTP binding protein)	2.21
<i>HOXA4</i>	homeobox A4	2.21
<i>ZMAT3</i>	zinc finger, matrin type 3	2.21
<i>VRK2</i>	vaccinia related kinase 2	2.21
<i>KIF2A</i>	kinesin heavy chain member 2A	2.20
<i>ZNF330</i>	zinc finger protein 330	2.20
<i>RAB9</i>	RAB9, member RAS oncogene family	2.19
<i>CAPZA1</i>	capping protein (actin filament) muscle Z-line, alpha 1	2.19
<i>FAM50A</i>	family with sequence similarity 50, member A	2.19
<i>PHTF1</i>	putative homeodomain transcription factor 1	2.19
<i>ATP6V1A</i>	ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A	2.19

Symbol	Gene name	Fold change
<i>POP4</i>	processing of precursor 4, ribonuclease P/MRP subunit (S. cerevisiae)	2.19
<i>IK</i>	IK cytokine, down-regulator of HLA II /// IK cytokine, down-regulator of HLA II	2.18
<i>HBS1L</i>	HBS1-like (S. cerevisiae)	2.18
<i>DCTN1</i>	dynactin 1 (p150, glued homolog, Drosophila)	2.18
<i>MARCKSL1</i>	MARCKS-like 1	2.18
<i>VPS4A</i>	vacuolar protein sorting 4 homolog A (S. cerevisiae)	2.17
<i>RAB1F</i>	RAB interacting factor	2.17
<i>CBX5</i>	chromobox homolog 5 (HP1 alpha homolog, Drosophila)	2.17
<i>IER3</i>	immediate early response 3	2.17
<i>PSMD1</i>	proteasome (prosome, macropain) 26S subunit, non-ATPase, 1	2.17
<i>WDR13</i>	WD repeat domain 13	2.17
<i>STRN4</i>	striatin, calmodulin binding protein 4	2.17
<i>RNF7</i>	ring finger protein 7	2.17
<i>PSMC5</i>	proteasome (prosome, macropain) 26S subunit, ATPase, 5	2.17
<i>CTNNA1</i>	catenin (cadherin-associated protein), alpha 1, 102kDa	2.17
<i>TMBIM1</i>	transmembrane BAX inhibitor motif containing 1	2.16
<i>HSPA4L</i>	heat shock 70kDa protein 4-like	2.16
<i>TXNL1</i>	thioredoxin-like 1	2.16
<i>TESK1</i>	testis-specific kinase 1	2.16
<i>HRAS</i>	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	2.16
<i>GSTO1</i>	glutathione S-transferase omega 1	2.16
<i>SDF2</i>	stromal cell-derived factor 2	2.16
<i>ANAPC10</i>	anaphase promoting complex subunit 10	2.16
<i>AKR1B10</i>	aldo-keto reductase family 1, member B10 (aldose reductase)	2.16
<i>DDX10</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 10	2.16
<i>ITSN2</i>	intersectin 2	2.15
<i>ACYP1</i>	acylphosphatase 1, erythrocyte (common) type	2.15
<i>BCKDK</i>	branched chain ketoacid dehydrogenase kinase	2.15
<i>MPP1</i>	membrane protein, palmitoylated 1, 55kDa	2.15
<i>ITGB1BP1</i>	integrin beta 1 binding protein 1	2.15
<i>STAM2</i>	signal transducing adaptor molecule (SH3 domain and ITAM motif) 2	2.14
<i>TMSB10</i>	thymosin, beta 10	2.14
<i>NR1I2</i>	nuclear receptor subfamily 1, group I, member 2	2.14
<i>NAT1</i>	N-acetyltransferase 1 (arylamine N-acetyltransferase)	2.14
<i>VAR5</i>	valyl-tRNA synthetase	2.14
<i>AP1G1</i>	adaptor-related protein complex 1, gamma 1 subunit	2.13
<i>ARPC1B</i> /// <i>LOC653888</i>	actin related protein 2/3 complex, subunit 1B, 41kDa /// similar to Actin-related protein 2/3 complex subunit 1B (ARP2/3 complex 41 kDa subunit) (p41-ARC)	2.13
<i>RAP2B</i>	RAP2B, member of RAS oncogene family	2.13
<i>DDX41</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 41	2.13
<i>SGCB</i>	sarcoglycan, beta (43kDa dystrophin-associated glycoprotein)	2.13
<i>MAP2K3</i>	mitogen-activated protein kinase kinase 3 /// mitogen-activated protein kinase kinase 3	2.13
<i>YY1</i>	YY1 transcription factor	2.13
<i>RPS6KA1</i>	ribosomal protein S6 kinase, 90kDa, polypeptide 1	2.13
<i>S100A13</i>	S100 calcium binding protein A13	2.13
<i>BACH1</i>	BTB and CNC homology 1, basic leucine zipper transcription factor 1	2.13
<i>BECN1</i>	beclin 1 (coiled-coil, myosin-like BCL2 interacting protein)	2.13
<i>ACSL1</i>	acyl-CoA synthetase long-chain family member 1	2.13
<i>RPS26</i> ///	ribosomal protein S26 /// similar to 40S ribosomal protein S26 /// similar to 40S	2.13

Symbol	Gene name	Fold change
<i>LOC644166</i> /// <i>LOC644191</i> /// <i>LOC728937</i>	ribosomal protein S26 /// similar to 40S ribosomal protein S26	
<i>SCPEP1</i>	serine carboxypeptidase 1	2.12
<i>GOLGA7</i>	golgi autoantigen, golgin subfamily a, 7	2.12
<i>ASMTL</i>	acetylserotonin O-methyltransferase-like	2.12
<i>RAB1B</i>	RAB1B, member RAS oncogene family /// RAB1B, member RAS oncogene family	2.11
<i>RNF11</i>	ring finger protein 11	2.11
<i>STAT1</i>	signal transducer and activator of transcription 1, 91kDa	2.11
<i>BAG2</i>	BCL2-associated athanogene 2	2.11
<i>USP11</i>	ubiquitin specific peptidase 11	2.10
<i>WNK1</i>	WNK lysine deficient protein kinase 1 /// WNK lysine deficient protein kinase 1	2.10
<i>MTCH1</i>	mitochondrial carrier homolog 1 (C. elegans)	2.10
<i>STX18</i>	syntaxin 18	2.10
<i>TPD52L2</i>	tumor protein D52-like 2	2.10
<i>MBD1</i>	methyl-CpG binding domain protein 1	2.09
<i>SNF8</i>	SNF8, ESCRT-II complex subunit, homolog (S. cerevisiae)	2.09
<i>STAT6</i>	signal transducer and activator of transcription 6, interleukin-4 induced	2.09
<i>ATXN2</i>	ataxin 2	2.07
<i>DAPK3</i>	death-associated protein kinase 3	2.07
<i>UBE2L3</i>	ubiquitin-conjugating enzyme E2L 3	2.07
<i>PSMC2</i>	proteasome (prosome, macropain) 26S subunit, ATPase, 2	2.07
<i>ARHGAP5</i>	Rho GTPase activating protein 5	2.07
<i>DYRK1A</i>	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	2.06
<i>ILK</i>	integrin-linked kinase	2.06
<i>PFDN1</i>	prefoldin subunit 1	2.06
<i>SEPW1</i>	selenoprotein W, 1	2.06
<i>PTPN14</i>	protein tyrosine phosphatase, non-receptor type 14	2.05
<i>WDR44</i>	WD repeat domain 44	2.05
<i>FAH</i>	fumarylacetoacetate hydrolase (fumarylacetoacetase)	2.05
<i>MIR16</i>	membrane interacting protein of RGS16	2.05
<i>GABPA</i> /// <i>GABPAP</i>	GA binding protein transcription factor, alpha subunit 60kDa /// GA binding protein transcription factor, alpha subunit pseudogene	2.05
<i>PGAM1</i> /// <i>LOC642969</i> /// <i>LOC643576</i>	phosphoglycerate mutase 1 (brain) /// similar to Phosphoglycerate mutase 1 (Phosphoglycerate mutase isozyme B) (PGAM-B) (BPG-dependent PGAM 1) /// similar to Phosphoglycerate mutase 1 (Phosphoglycerate mutase isozyme B) (PGAM-B) (BPG-dependent PGAM 1)	2.05
<i>RDBP</i>	RD RNA binding protein	2.05
<i>LYPLA1</i>	lysophospholipase I	2.04
<i>EIF3S2</i>	eukaryotic translation initiation factor 3, subunit 2 beta, 36kDa	2.04
<i>GBF1</i>	golgi-specific brefeldin A resistance factor 1	2.04
<i>CDC42</i>	cell division cycle 42 (GTP binding protein, 25kDa)	2.04
<i>ARPC3</i>	actin related protein 2/3 complex, subunit 3, 21kDa	2.04
<i>TOP1</i>	topoisomerase (DNA) I	2.04
<i>RGL2</i>	ral guanine nucleotide dissociation stimulator-like 2	2.04
<i>TFCP2</i>	transcription factor CP2	2.03
<i>PSMB3</i>	proteasome (prosome, macropain) subunit, beta type, 3	2.03
<i>PPP2CA</i>	protein phosphatase 2 (formerly 2A), catalytic subunit, alpha isoform	2.03
<i>BCL10</i>	B-cell CLL/lymphoma 10	2.03
<i>ATG5</i>	ATG5 autophagy related 5 homolog (S. cerevisiae)	2.03

Symbol	Gene name	Fold change
<i>DAZAP2</i>	DAZ associated protein 2	2.03
<i>LRRFIP2</i>	leucine rich repeat (in FLII) interacting protein 2	2.03
<i>LTBR</i>	lymphotoxin beta receptor (TNFR superfamily, member 3)	2.03
<i>GRSF1</i>	G-rich RNA sequence binding factor 1	2.02
<i>WBP11</i>	WW domain binding protein 11	2.02
<i>GSS</i>	glutathione synthetase	2.02
<i>GNS</i>	glucosamine (N-acetyl)-6-sulfatase (Sanfilippo disease IIID)	2.02
<i>THRAP5</i>	thyroid hormone receptor associated protein 5	2.01
<i>MXI1</i>	MAX interactor 1 /// MAX interactor 1	2.01
<i>OSTF1</i>	osteoclast stimulating factor 1	2.01
<i>MYL6</i>	myosin, light chain 6, alkali, smooth muscle and non-muscle	2.01
<i>EGLN1</i>	egl nine homolog 1 (C. elegans)	2.01
<i>CASP8</i>	caspase 8, apoptosis-related cysteine peptidase	2.01
<i>PIK3CB</i>	phosphoinositide-3-kinase, catalytic, beta polypeptide	2.01
<i>ERCC3</i>	excision repair cross-complementing rodent repair deficiency, complementation group 3 (xeroderma pigmentosum group B complementing)	2.01
<i>NEDD8</i>	neural precursor cell expressed, developmentally down-regulated 8	2.01

5 b) Down-regulated genes in 40 µM exposure

Symbol	Gene name	Fold change
<i>FGB</i>	fibrinogen beta chain	105.88
<i>FGA</i>	fibrinogen alpha chain	64.05
<i>CPB2</i>	carboxypeptidase B2 (plasma, carboxypeptidase U)	57.59
<i>CP</i>	ceruloplasmin (ferroxidase)	51.24
<i>SERPINA3</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	43.13
<i>RNASE4</i>	ribonuclease, RNase A family, 4	36.83
<i>ASGR1</i>	asialoglycoprotein receptor 1	30.31
<i>CCL20</i>	chemokine (C-C motif) ligand 20	30.16
<i>APOM</i>	apolipoprotein M	29.61
<i>FGG</i>	fibrinogen gamma chain	27.36
<i>TTR</i>	transthyretin (prealbumin, amyloidosis type I)	25.07
<i>PAH</i>	phenylalanine hydroxylase	23.67
<i>FMO5</i>	flavin containing monooxygenase 5	23.33
<i>DNAJC12</i>	DnaJ (Hsp40) homolog, subfamily C, member 12	21.75
<i>ORM1 /// ORM2</i>	orosomucoid 1 /// orosomucoid 2	19.54
<i>SERPINA5</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5	19.35
<i>CPN1</i>	carboxypeptidase N, polypeptide 1, 50kD	18.49
<i>SERPIND1</i>	serpin peptidase inhibitor, clade D (heparin cofactor), member 1	18.26
<i>TGFB3</i>	transforming growth factor, beta receptor III (betaglycan, 300kDa)	18.22
<i>C5</i>	complement component 5	17.95
<i>SEPP1</i>	selenoprotein P, plasma, 1	17.18
<i>AGT</i>	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	16.21
<i>IL1RN</i>	interleukin 1 receptor antagonist	16.00
<i>IGSF1</i>	immunoglobulin superfamily, member 1	15.10
<i>HIST1H4C</i>	histone cluster 1, H4c	15.06
<i>TNFSF10</i>	tumor necrosis factor (ligand) superfamily, member 10 /// tumor necrosis factor (ligand) superfamily, member 10	14.94
<i>SOX9</i>	SRY (sex determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	14.15
<i>ORM1 /// ORM2</i>	orosomucoid 1 /// orosomucoid 2	13.30
<i>SERPINC1</i>	serpin peptidase inhibitor, clade C (antithrombin), member 1	12.89
<i>HLF</i>	hepatic leukemia factor	12.40
<i>RGN</i>	regucalcin (senescence marker protein-30)	12.00
<i>LOX</i>	lysyl oxidase	11.96
<i>CFI</i>	complement factor I	11.94
<i>ALCAM</i>	activated leukocyte cell adhesion molecule	11.79
<i>F5</i>	coagulation factor V (proaccelerin, labile factor)	11.76
<i>FGFR4</i>	fibroblast growth factor receptor 4	11.63
<i>IGSF4</i>	immunoglobulin superfamily, member 4	11.51
<i>LGR5</i>	leucine-rich repeat-containing G protein-coupled receptor 5	11.41
<i>CFB</i>	complement factor B	11.10
<i>F11</i>	coagulation factor XI (plasma thromboplastin antecedent)	10.88
<i>HYAL1</i>	hyaluronoglucosaminidase 1	10.49
<i>DUSP9</i>	dual specificity phosphatase 9	10.37
<i>B3GNT1</i>	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1	10.14
<i>GLT8D1</i>	glycosyltransferase 8 domain containing 1	9.99
<i>ID1</i>	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	9.88
<i>ST6GAL1</i>	ST6 beta-galactosamide alpha-2,6-sialyltransferase 1	9.87

Symbol	Gene name	Fold change
<i>SNX5</i>	sorting nexin 5	9.87
<i>DDC</i>	dopa decarboxylase (aromatic L-amino acid decarboxylase)	9.86
<i>SC4MOL</i>	sterol-C4-methyl oxidase-like	9.43
<i>GAS2</i>	growth arrest-specific 2	9.41
<i>A2M</i>	alpha-2-macroglobulin	9.33
<i>ASPSCR1</i>	alveolar soft part sarcoma chromosome region, candidate 1	9.31
<i>ALDH18A1</i>	aldehyde dehydrogenase 18 family, member A1	9.21
<i>TRIM15</i>	tripartite motif-containing 15	9.02
<i>VIL1</i>	villin 1	8.88
<i>ARSE</i>	arylsulfatase E (chondrodysplasia punctata 1)	8.82
<i>MYO10</i>	myosin X	8.81
<i>PGC</i>	progastricsin (pepsinogen C)	8.75
<i>DHCR7</i>	7-dehydrocholesterol reductase	8.75
<i>C4BPB</i>	complement component 4 binding protein, beta	8.73
<i>MAGED1</i>	melanoma antigen family D, 1	8.70
<i>SORL1</i>	soritin-related receptor, L(DLR class) A repeats-containing	8.57
<i>CDH2</i>	cadherin 2, type 1, N-cadherin (neuronal)	8.50
<i>ERBB3</i>	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	8.50
<i>APOH</i>	apolipoprotein H (beta-2-glycoprotein I)	8.47
<i>EFNA1</i>	ephrin-A1	7.99
<i>APOA1</i>	apolipoprotein A-I	7.86
<i>RABEPK</i>	Rab9 effector protein with kelch motifs	7.81
<i>RPL31</i> /// <i>LOC285260</i> /// <i>RPL31P4</i> /// <i>RPL31P10</i> /// <i>LOC641790</i> /// <i>LOC646841</i> /// <i>LOC648737</i> /// <i>LOC653773</i> /// <i>LOC727792</i> /// <i>LOC729646</i> /// <i>LOC732015</i>	ribosomal protein L31 /// similar to ribosomal protein L31 /// ribosomal protein L31 pseudogene 4 /// ribosomal protein L31 pseudogene 10 /// similar to ribosomal protein L31 /// similar to ribosomal protein L31 /// similar to ribosomal protein L31 /// similar to ribosomal protein L31 /// similar to ribosomal protein L31 /// hypothetical protein LOC729646 /// similar to ribosomal protein L31	7.69
<i>CLDN1</i>	claudin 1	7.43
<i>F10</i>	coagulation factor X	7.22
<i>AHSG</i>	alpha-2-HS-glycoprotein	7.20
<i>ANPEP</i>	alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)	7.05
<i>BRP44L</i>	brain protein 44-like	6.94
<i>CX3CL1</i>	chemokine (C-X3-C motif) ligand 1	6.87
<i>SERPINF1</i>	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	6.83
<i>ASS1</i>	argininosuccinate synthetase 1	6.78
<i>HMGCGR</i>	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	6.77
<i>FDFT1</i>	farnesyl-diphosphate farnesyltransferase 1	6.75
<i>CXADR</i>	coxsackie virus and adenovirus receptor	6.74
<i>AGTR1</i>	angiotensin II receptor, type 1	6.68
<i>NR0B2</i>	nuclear receptor subfamily 0, group B, member 2	6.63
<i>ITIH3</i>	inter-alpha (globulin) inhibitor H3	6.62
<i>AKR1D1</i>	aldo-keto reductase family 1, member D1 (delta 4-3-ketosteroid-5-beta-reductase)	6.58

Symbol	Gene name	Fold change
<i>APOB</i>	apolipoprotein B (including Ag(x) antigen)	6.56
<i>PROC</i>	protein C (inactivator of coagulation factors Va and VIIIa)	6.55
<i>PTPRK</i>	protein tyrosine phosphatase, receptor type, K	6.54
<i>NEDD9</i>	neural precursor cell expressed, developmentally down-regulated 9	6.41
<i>LIPA</i>	lipase A, lysosomal acid, cholesterol esterase (Wolman disease)	6.40
<i>MGST2</i>	microsomal glutathione S-transferase 2	6.39
<i>PHGDH</i>	phosphoglycerate dehydrogenase	6.31
<i>PRIM1</i>	primase, polypeptide 1, 49kDa	6.27
<i>C10orf10</i>	chromosome 10 open reading frame 10	6.26
<i>HSP90B1</i>	heat shock protein 90kDa beta (Grp94), member 1	6.20
<i>TST</i>	thiosulfate sulfurtransferase (rhodanese)	6.19
<i>DPYD</i>	dihydropyrimidine dehydrogenase	6.19
<i>RAPGEF5</i>	Rap guanine nucleotide exchange factor (GEF) 5	6.17
<i>IL17RB</i>	interleukin 17 receptor B	6.15
<i>FGFR3</i>	fibroblast growth factor receptor 3 (achondroplasia, thanatophoric dwarfism)	6.01
<i>PCSK6</i>	proprotein convertase subtilisin/kexin type 6	6.00
<i>RELN</i>	reelin	5.91
<i>GLUD1</i>	glutamate dehydrogenase 1	5.89
<i>COX11</i>	COX11 homolog, cytochrome c oxidase assembly protein (yeast)	5.87
<i>ACOT2</i> /// <i>ACOT1</i>	acyl-CoA thioesterase 2 /// acyl-CoA thioesterase 1	5.87
<i>SCD</i>	stearoyl-CoA desaturase (delta-9-desaturase)	5.84
<i>C2</i>	complement component 2	5.84
<i>C6orf48</i>	chromosome 6 open reading frame 48	5.79
<i>ABCB4</i>	ATP-binding cassette, sub-family B (MDR/TAP), member 4	5.78
<i>FST</i>	folistatin	5.77
	Full-length cDNA clone CS0DC014YA20 of Neuroblastoma Cot 25-normalized of Homo sapiens (human)	5.75
<i>HHEX</i>	homeobox, hematopoietically expressed	5.71
<i>CCDC85B</i>	coiled-coil domain containing 85B	5.68
<i>SLC2A10</i>	solute carrier family 2 (facilitated glucose transporter), member 10 /// solute carrier family 2 (facilitated glucose transporter), member 10	5.64
<i>PGRMC2</i>	progesterone receptor membrane component 2	5.59
<i>HPN</i>	hepsin (transmembrane protease, serine 1)	5.59
<i>MEST</i>	mesoderm specific transcript homolog (mouse)	5.58
<i>VAV3</i>	vav 3 oncogene	5.57
<i>PSAT1</i>	phosphoserine aminotransferase 1	5.55
<i>P2RY5</i>	purinergic receptor P2Y, G-protein coupled, 5	5.53
<i>C4A</i> /// <i>C4B</i>	complement component 4A (Rodgers blood group) /// complement component 4B (Childo blood group)	5.52
<i>LOC653879</i>	similar to Complement C3 precursor	5.51
<i>SQLE</i>	squalene epoxidase	5.49
<i>TF</i>	transferrin	5.46
<i>EML4</i>	echinoderm microtubule associated protein like 4	5.37
<i>PIPOX</i>	pipecolic acid oxidase	5.37
<i>HMGS1</i>	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	5.34
<i>NASP</i>	nuclear autoantigenic sperm protein (histone-binding)	5.29
<i>VTN</i>	vitronectin	5.27
<i>NDRG2</i>	NDRG family member 2	5.26
<i>PECR</i>	peroxisomal trans-2-enoyl-CoA reductase	5.21

Symbol	Gene name	Fold change
<i>IFITM3</i>	interferon induced transmembrane protein 3 (1-8U)	5.21
<i>PER2</i>	period homolog 2 (Drosophila)	5.20
<i>FOXA2</i>	forkhead box A2	5.19
<i>IFITM2</i>	interferon induced transmembrane protein 2 (1-8D)	5.15
<i>CRLF1</i>	cytokine receptor-like factor 1	5.13
<i>MYC</i>	v-myc myelocytomatosis viral oncogene homolog (avian)	5.13
<i>ACF</i>	apobec-1 complementation factor	5.11
<i>EIF1AX</i>	eukaryotic translation initiation factor 1A, X-linked	5.10
<i>SLC25A15</i>	solute carrier family 25 (mitochondrial carrier; ornithine transporter) member 15	5.07
<i>TIA1</i>	TIA1 cytotoxic granule-associated RNA binding protein	5.07
<i>ENPP1</i>	ectonucleotide pyrophosphatase/phosphodiesterase 1	5.05
<i>ENC1</i>	ectodermal-neural cortex (with BTB-like domain)	5.05
<i>SMARCE1</i>	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily e, member 1	5.04
<i>ALDH1B1</i>	aldehyde dehydrogenase 1 family, member B1	5.02
<i>CCNB1IP1</i>	cyclin B1 interacting protein 1	4.96
<i>IMPA2</i>	inositol(myo)-1(or 4)-monophosphatase 2	4.95
<i>CIDEB</i>	cell death-inducing DFFA-like effector b	4.95
<i>EPHB4</i>	EPH receptor B4	4.94
<i>DHCR24</i>	24-dehydrocholesterol reductase	4.91
<i>ALDH1A1</i>	aldehyde dehydrogenase 1 family, member A1	4.90
<i>GPX2</i>	glutathione peroxidase 2 (gastrointestinal)	4.80
<i>SAA4</i>	serum amyloid A4, constitutive	4.80
<i>PDIA4</i>	protein disulfide isomerase family A, member 4	4.77
<i>C1RL</i>	complement component 1, r subcomponent-like	4.76
<i>PSPH</i>	phosphoserine phosphatase	4.76
<i>SLC37A4</i>	solute carrier family 37 (glycerol-6-phosphate transporter), member 4	4.70
<i>RAB17</i>	RAB17, member RAS oncogene family	4.69
<i>CCL16</i>	chemokine (C-C motif) ligand 16	4.63
<i>RAB40B</i>	RAB40B, member RAS oncogene family	4.63
<i>APOC2</i>	apolipoprotein C-II	4.63
<i>ITIH2</i>	inter-alpha (globulin) inhibitor H2	4.63
<i>ASNS</i>	asparagine synthetase	4.59
<i>SPHAR</i>	S-phase response (cyclin-related)	4.59
<i>TM7SF2</i>	transmembrane 7 superfamily member 2	4.58
<i>SLC27A2</i>	solute carrier family 27 (fatty acid transporter), member 2	4.56
<i>TNFAIP3</i>	tumor necrosis factor, alpha-induced protein 3	4.47
<i>ECH1</i>	enoyl Coenzyme A hydratase 1, peroxisomal	4.39
<i>USP13</i>	ubiquitin specific peptidase 13 (isopeptidase T-3)	4.39
<i>GLUD2</i>	glutamate dehydrogenase 2	4.34
<i>MMD</i>	monocyte to macrophage differentiation-associated	4.34
<i>ALDH6A1</i>	aldehyde dehydrogenase 6 family, member A1	4.32
<i>SORBS1</i>	sorbin and SH3 domain containing 1	4.32
<i>IKBKAP</i>	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein	4.32
<i>SGCE</i>	sarcoglycan, epsilon	4.31
<i>F2</i>	coagulation factor II (thrombin)	4.28
<i>SHMT2</i>	serine hydroxymethyltransferase 2 (mitochondrial)	4.24
<i>LMNB1</i>	lamin B1	4.24

Symbol	Gene name	Fold change
<i>LOC730013</i>	similar to ATP-binding cassette, sub-family C, member 6	4.22
<i>HMG2A</i>	high mobility group AT-hook 2 /// high mobility group AT-hook 2	4.22
<i>SNTB1</i>	syntrophin, beta 1 (dystrophin-associated protein A1, 59kDa, basic component 1)	4.22
<i>CDC2</i>	cell division cycle 2, G1 to S and G2 to M	4.18
<i>SLC2A4RG</i>	SLC2A4 regulator	4.17
<i>PCK2</i>	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	4.17
<i>BAX</i>	BCL2-associated X protein	4.16
<i>DLEU1 /// SPANXC</i>	deleted in lymphocytic leukemia, 1 /// SPANX family, member C	4.14
<i>PAICS</i>	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase	4.13
<i>NPM3</i>	nucleophosmin/nucleoplamin, 3	4.11
<i>CYP51A1</i>	cytochrome P450, family 51, subfamily A, polypeptide 1	4.10
<i>IQGAP2</i>	IQ motif containing GTPase activating protein 2	4.09
<i>GNG4</i>	guanine nucleotide binding protein (G protein), gamma 4	4.09
<i>ITPR2</i>	inositol 1,4,5-triphosphate receptor, type 2	4.08
<i>HNMT</i>	histamine N-methyltransferase	4.07
<i>RBP4</i>	retinol binding protein 4, plasma	4.07
<i>RPL15</i>	ribosomal protein L15	4.04
<i>AFP</i>	alpha-fetoprotein	4.03
<i>CTSH</i>	cathepsin H	4.00
<i>FDXR</i>	ferredoxin reductase	3.99
<i>RAB4A</i>	RAB4A, member RAS oncogene family	3.99
<i>RXRA</i>	retinoid X receptor, alpha	3.96
<i>APEX1</i>	APEX nuclease (multifunctional DNA repair enzyme) 1	3.93
<i>PCYOX1</i>	prenylcysteine oxidase 1	3.93
<i>PLD1</i>	phospholipase D1, phosphatidylcholine-specific	3.91
<i>FZD4</i>	frizzled homolog 4 (Drosophila)	3.90
<i>BET1</i>	BET1 homolog (S. cerevisiae)	3.88
<i>TOMM20</i>	translocase of outer mitochondrial membrane 20 homolog (yeast)	3.86
<i>GCHFR</i>	GTP cyclohydrolase I feedback regulator	3.85
<i>FRAT1</i>	frequently rearranged in advanced T-cell lymphomas	3.85
<i>SHMT2</i>	serine hydroxymethyltransferase 2 (mitochondrial)	3.85
<i>PDIA5</i>	protein disulfide isomerase family A, member 5	3.84
<i>PDIA6</i>	protein disulfide isomerase family A, member 6	3.83
<i>CTSC</i>	cathepsin C	3.82
<i>PIP5K1B</i>	phosphatidylinositol-4-phosphate 5-kinase, type I, beta	3.81
<i>TSPAN6</i>	tetraspanin 6	3.79
<i>SLC9A3R1</i>	solute carrier family 9 (sodium/hydrogen exchanger), member 3 regulator 1	3.77
<i>GIN52</i>	GIN5 complex subunit 2 (Psf2 homolog)	3.75
<i>ROBO1</i>	roundabout, axon guidance receptor, homolog 1 (Drosophila)	3.75
<i>GRB7</i>	growth factor receptor-bound protein 7	3.74
<i>DSP</i>	desmoplakin	3.74
<i>ETFB</i>	electron-transfer-flavoprotein, beta polypeptide	3.74
<i>PSPH</i>	phosphoserine phosphatase	3.72
<i>RPL10</i>	ribosomal protein L10	3.72
<i>GBAS</i>	glioblastoma amplified sequence	3.70
<i>ZNF331</i>	zinc finger protein 331	3.70
<i>SYNCRIP</i>	synaptotagmin binding, cytoplasmic RNA interacting protein	3.69

Symbol	Gene name	Fold change
AMACR	alpha-methylacyl-CoA racemase	3.65
NTHL1	nth endonuclease III-like 1 (E. coli)	3.65
PEBP1	phosphatidylethanolamine binding protein 1	3.65
ACSM3	acyl-CoA synthetase medium-chain family member 3	3.63
CPD	carboxypeptidase D	3.61
HAGH	hydroxyacylglutathione hydrolase	3.61
CELSR2	cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, Drosophila)	3.59
NCAPG	non-SMC condensin I complex, subunit G	3.58
FDPS	farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallyltranstransferase, geranyltranstransferase)	3.57
PEMT	phosphatidylethanolamine N-methyltransferase	3.54
C2orf28	chromosome 2 open reading frame 28	3.53
PPAP2B	phosphatidic acid phosphatase type 2B	3.50
CYB5A	cytochrome b5 type A (microsomal)	3.50
HSD17B11	hydroxysteroid (17-beta) dehydrogenase 11	3.50
ATP7B	ATPase, Cu++ transporting, beta polypeptide	3.49
MUT	methyalmalonyl Coenzyme A mutase	3.48
RANBP1	RAN binding protein 1	3.48
HIST1H3H	histone cluster 1, H3h	3.48
CES2	carboxylesterase 2 (intestine, liver)	3.47
PROM1	prominin 1	3.46
MRPL23	mitochondrial ribosomal protein L23	3.43
ACAA2	acetyl-Coenzyme A acyltransferase 2 (mitochondrial 3-oxoacyl-Coenzyme A thiolase)	3.43
TRAP1	TNF receptor-associated protein 1	3.41
WWTR1	WW domain containing transcription regulator 1	3.40
ILVBL	ilvB (bacterial acetolactate synthase)-like	3.39
TACSTD1	tumor-associated calcium signal transducer 1	3.39
AHR	aryl hydrocarbon receptor	3.38
TACC1	transforming, acidic coiled-coil containing protein 1	3.38
KNTC2	kinetochore associated 2	3.38
CA5A	carbonic anhydrase VA, mitochondrial	3.36
SCML1	sex comb on midleg-like 1 (Drosophila)	3.36
FKBP11	FK506 binding protein 11, 19 kDa	3.36
CIRBP	cold inducible RNA binding protein	3.31
IL1RAP	interleukin 1 receptor accessory protein	3.29
TOP2A	topoisomerase (DNA) II alpha 170kDa	3.29
ABCD4	ATP-binding cassette, sub-family D (ALD), member 4	3.27
DCXR	dicarbonyl/L-xylulose reductase	3.25
RBMX	RNA binding motif protein, X-linked	3.25
MCM3	MCM3 minichromosome maintenance deficient 3 (S. cerevisiae)	3.23
CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)	3.23
RFC4	replication factor C (activator 1) 4, 37kDa	3.23
HSD17B4	hydroxysteroid (17-beta) dehydrogenase 4	3.23
FADS1	fatty acid desaturase 1	3.22
LPHN2	latrophilin 2	3.22
MAN1A1	Mannosidase, alpha, class 1A, member 1	3.22
CPVL	carboxypeptidase, vitellogenic-like /// carboxypeptidase, vitellogenic-like	3.20
GRHPR	glyoxylate reductase/hydroxypyruvate reductase	3.20
MPST	mercaptopyruvate sulfurtransferase	3.20

Symbol	Gene name	Fold change
DTYMK /// LOC727761	deoxythymidylate kinase (thymidylate kinase) /// similar to deoxythymidylate kinase (thymidylate kinase)	3.19
XBP1	X-box binding protein 1	3.19
MYO5C	myosin VC	3.19
SLC19A1	solute carrier family 19 (folate transporter), member 1	3.18
NR2F1	Nuclear receptor subfamily 2, group F, member 1	3.17
DICER1	Dicer1, Dcr-1 homolog (Drosophila)	3.17
RNF13	ring finger protein 13	3.17
DPM3	dolichyl-phosphate mannosyltransferase polypeptide 3	3.16
TFPI	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	3.15
PCGF2	polycomb group ring finger 2	3.14
C14orf1	chromosome 14 open reading frame 1	3.14
GPC3	glypican 3	3.14
SERPINA1	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	3.13
EBP	emopamil binding protein (sterol isomerase)	3.13
FILIP1L	filamin A interacting protein 1-like	3.13
GSTK1	glutathione S-transferase kappa 1	3.12
ACSL3	acyl-CoA synthetase long-chain family member 3	3.10
HMMR	hyaluronan-mediated motility receptor (RHAMM)	3.08
HGD	homogentisate 1,2-dioxygenase (homogentisate oxidase)	3.07
CDC25C	cell division cycle 25 homolog C (S. cerevisiae)	3.07
ABCB6	ATP-binding cassette, sub-family B (MDR/TAP), member 6	3.06
MAT1A	methionine adenosyltransferase I, alpha	3.06
ALDH3A2	aldehyde dehydrogenase 3 family, member A2	3.04
GLDC	glycine dehydrogenase (decarboxylating)	3.04
PBK	PDZ binding kinase	3.03
AZGP1	alpha-2-glycoprotein 1, zinc-binding	3.03
AKAP7	A kinase (PRKA) anchor protein 7	3.02
NIPSNAP1	nipsnap homolog 1 (C. elegans)	3.02
CD74	CD74 molecule, major histocompatibility complex, class II invariant chain	3.00
HSD17B7 /// HSD17B7P2 /// LOC730412	hydroxysteroid (17-beta) dehydrogenase 7 /// hydroxysteroid (17-beta) dehydrogenase 7 pseudogene 2 /// similar to hydroxysteroid (17-beta) dehydrogenase 7	2.99
LGR4	leucine-rich repeat-containing G protein-coupled receptor 4	2.99
EZH2	enhancer of zeste homolog 2 (Drosophila)	2.99
NID1	nidogen 1	2.98
AGA	aspartylglucosaminidase	2.97
BTN3A3 /// BTN3A2	butyrophilin, subfamily 3, member A3 /// butyrophilin, subfamily 3, member A2	2.96
CETN3	centrin, EF-hand protein, 3 (CDC31 homolog, yeast)	2.96
ALDH7A1	aldehyde dehydrogenase 7 family, member A1	2.96
B4GALT4	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 4	2.96
EPB41L2	erythrocyte membrane protein band 4.1-like 2	2.96
KIF20A	kinesin family member 20A	2.95
LTA4H	leukotriene A4 hydrolase	2.95
GSN	gelsolin (amyloidosis, Finnish type)	2.95
IDH1	isocitrate dehydrogenase 1 (NADP+), soluble	2.94
NUDT21	nudix (nucleoside diphosphate linked moiety X)-type motif 21	2.94
SOX12	SRY (sex determining region Y)-box 12	2.93

Symbol	Gene name	Fold change
<i>ATPIF1</i>	ATPase inhibitory factor 1	2.93
<i>PCNP</i>	PEST proteolytic signal containing nuclear protein	2.93
<i>PAK1 /// VDP</i>	p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast) /// vesicle docking protein p115	2.92
<i>MAP2K6</i>	mitogen-activated protein kinase kinase 6	2.92
<i>RABGGTB</i>	Rab geranylgeranyltransferase, beta subunit	2.91
<i>ITGB2</i>	integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	2.91
<i>MTHFD2</i>	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase	2.91
<i>MCCC2</i>	methylcrotonoyl-Coenzyme A carboxylase 2 (beta)	2.90
<i>ZNF217</i>	zinc finger protein 217	2.89
<i>KCTD3</i>	potassium channel tetramerisation domain containing 3	2.89
<i>PPP3CB</i>	protein phosphatase 3 (formerly 2B), catalytic subunit, beta isoform (calcineurin A beta)	2.89
<i>ADH5</i>	alcohol dehydrogenase 5 (class III), chi polypeptide	2.89
<i>PPIB</i>	peptidylprolyl isomerase B (cyclophilin B)	2.89
<i>CCNA2</i>	cyclin A2	2.88
<i>MAGED4</i>	melanoma antigen family D, 4 /// melanoma antigen family D, 4	2.88
<i>KLHDC2</i>	kelch domain containing 2	2.88
<i>SFRP4</i>	secreted frizzled-related protein 4	2.87
<i>LIMK2</i>	LIM domain kinase 2	2.87
<i>BMPR1A</i>	bone morphogenetic protein receptor, type IA	2.87
<i>HMBS</i>	hydroxymethylbilane synthase	2.86
<i>CD46</i>	CD46 molecule, complement regulatory protein	2.86
<i>ARL6IP4</i>	ADP-ribosylation-like factor 6 interacting protein 4	2.86
<i>MAN1B1</i>	mannosidase, alpha, class 1B, member 1	2.85
<i>CALD1</i>	caldesmon 1	2.85
<i>SC5DL</i>	sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, fungal)-like	2.83
<i>TTC3</i>	tetratricopeptide repeat domain 3	2.82
<i>CCNB2</i>	cyclin B2	2.81
<i>PEBP1</i>	phosphatidylethanolamine binding protein 1	2.80
<i>EBAG9</i>	estrogen receptor binding site associated, antigen, 9	2.80
<i>CLGN</i>	calmegin	2.80
<i>ZWINT</i>	ZW10 interactor	2.80
<i>PMS1</i>	PMS1 postmeiotic segregation increased 1 (S. cerevisiae)	2.80
<i>MCM5</i>	MCM5 minichromosome maintenance deficient 5, cell division cycle 46 (S. cerevisiae)	2.79
<i>SERPINB1</i>	serpin peptidase inhibitor, clade B (ovalbumin), member 1	2.79
<i>SCMH1</i>	sex comb on midleg homolog 1 (Drosophila)	2.78
<i>PDSS1</i>	prenyl (decaprenyl) diphosphate synthase, subunit 1	2.78
<i>GK</i>	glycerol kinase	2.78
<i>ANAPC1</i>	anaphase promoting complex subunit 1	2.77
<i>PRMT1</i>	protein arginine methyltransferase 1	2.77
<i>CENPA</i>	centromere protein A	2.76
<i>PTCH1</i>	patched homolog 1 (Drosophila)	2.76
<i>SESN1</i>	sestrin 1	2.74
<i>ERBB2</i>	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	2.74
<i>ARL2</i>	ADP-ribosylation factor-like 2	2.74
<i>ACADM</i>	acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain	2.73

Symbol	Gene name	Fold change
<i>DPYSL2</i>	dihydropyrimidinase-like 2	2.73
<i>BRD3</i>	bromodomain containing 3	2.73
<i>TEX264</i>	testis expressed sequence 264	2.73
<i>FARSLA</i>	phenylalanine-tRNA synthetase-like, alpha subunit	2.72
<i>RAD51AP1</i>	RAD51 associated protein 1	2.72
<i>PRPSAP1</i>	phosphoribosyl pyrophosphate synthetase-associated protein 1	2.72
<i>TARBP1</i>	Tar (HIV-1) RNA binding protein 1	2.72
<i>RPL31</i>	ribosomal protein L31	2.71
<i>WDR57</i>	WD repeat domain 57 (U5 snRNP specific)	2.71
<i>PHYH</i>	phytanoyl-CoA 2-hydroxylase	2.71
<i>PDPK1</i>	3-phosphoinositide dependent protein kinase-1	2.71
<i>CPT2</i>	carnitine palmitoyltransferase II	2.70
<i>C1orf25</i>	chromosome 1 open reading frame 25 /// chromosome 1 open reading frame 25	2.70
<i>CALR</i>	Calreticulin	2.69
<i>BIRC5</i>	baculoviral IAP repeat-containing 5 (survivin)	2.69
<i>RPL41</i>	Ribosomal protein L41	2.69
<i>PLLP</i>	plasma membrane proteolipid (plasmolipin)	2.69
<i>LEPRE1</i>	leucine proline-enriched proteoglycan (leprecan) 1	2.69
<i>RPS9</i>	ribosomal protein S9	2.68
<i>GNE</i>	glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase	2.68
<i>PUS1</i>	pseudouridylate synthase 1	2.68
<i>YEATS4</i>	YEATS domain containing 4	2.67
<i>ATP6VOE2</i>	ATPase, H+ transporting VO subunit e2	2.66
<i>TGFB2</i>	transforming growth factor, beta receptor II (70/80kDa)	2.65
<i>GNPAT</i>	glyceronephosphate O-acyltransferase	2.65
<i>AARS</i>	alanyl-tRNA synthetase	2.65
<i>CHN2</i>	chimerin (chimaerin) 2	2.64
<i>DECR2</i>	2,4-dienoyl CoA reductase 2, peroxisomal	2.64
<i>SLC16A1</i>	solute carrier family 16, member 1 (monocarboxylic acid transporter 1)	2.64
<i>ALDH4A1</i>	aldehyde dehydrogenase 4 family, member A1	2.63
<i>BTN3A2</i>	butyrophilin, subfamily 3, member A2	2.62
<i>PYCR1</i>	pyrroline-5-carboxylate reductase 1	2.62
<i>MBTPS1</i>	membrane-bound transcription factor peptidase, site 1	2.62
<i>CAST</i>	calpastatin	2.62
<i>PLK1</i>	polo-like kinase 1 (Drosophila)	2.60
<i>MRS2L</i>	MRS2-like, magnesium homeostasis factor (S. cerevisiae)	2.60
<i>FXN</i>	frataxin	2.58
<i>PUM1</i>	pumilio homolog 1 (Drosophila)	2.58
<i>IL22RA1</i>	interleukin 22 receptor, alpha 1	2.58
<i>2-Sep</i>	septin 2 /// septin 2	2.57
<i>GSTA4</i>	glutathione S-transferase A4	2.57
<i>LRPAP1</i>	low density lipoprotein receptor-related protein associated protein 1	2.57
<i>WWOX</i>	WW domain containing oxidoreductase	2.57
<i>SMARCA5</i>	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5	2.56
<i>LAMB2</i>	laminin, beta 2 (laminin S)	2.56
<i>PLK4</i>	polo-like kinase 4 (Drosophila)	2.56
<i>H2AFV</i>	H2A histone family, member V	2.55
<i>ACAA1</i>	acetyl-Coenzyme A acyltransferase 1 (peroxisomal 3-oxoacyl-Coenzyme A thiolase)	2.54

Symbol	Gene name	Fold change
<i>LGTN</i>	ligatin	2.54
<i>NFAT5</i>	nuclear factor of activated T-cells 5, tonicity-responsive	2.54
<i>ACACA</i>	acetyl-Coenzyme A carboxylase alpha	2.54
<i>TBL2</i>	transducin (beta)-like 2	2.54
<i>DDB2</i>	damage-specific DNA binding protein 2, 48kDa	2.53
<i>SF3A3</i>	splicing factor 3a, subunit 3, 60kDa	2.53
<i>MINPP1</i>	multiple inositol polyphosphate histidine phosphatase, 1	2.52
<i>COMT</i>	catechol-O-methyltransferase	2.52
<i>PYGL</i>	phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI)	2.52
<i>GOLGA5</i>	golgi autoantigen, golgin subfamily a, 5	2.52
<i>UBE4B</i>	ubiquitination factor E4B (UFD2 homolog, yeast)	2.52
<i>NONO</i>	non-POU domain containing, octamer-binding /// non-POU domain containing, octamer-binding	2.51
<i>ACP6</i>	acid phosphatase 6, lysophosphatidic	2.51
<i>SLC12A7</i>	solute carrier family 12 (potassium/chloride transporters), member 7	2.51
<i>ARID3A</i>	AT rich interactive domain 3A (BRIGHT-like)	2.51
<i>IVD</i>	isovaleryl Coenzyme A dehydrogenase	2.50
<i>MTHFD1</i>	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1, methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase	2.50
<i>RANBP5</i>	RAN binding protein 5	2.49
<i>PGM1</i>	phosphoglucumutase 1	2.49
<i>SEC63</i>	SEC63 homolog (S. cerevisiae)	2.49
<i>KPNB1</i>	Karyopherin (importin) beta 1	2.49
<i>MAGED2</i>	melanoma antigen family D, 2	2.49
<i>HYOU1</i>	hypoxia up-regulated 1	2.49
<i>EXOSC5</i>	exosome component 5	2.48
<i>PSIP1</i>	PC4 and SFRS1 interacting protein 1	2.48
<i>PCOLCE2</i>	procollagen C-endopeptidase enhancer 2	2.47
<i>MAD2L1</i>	MAD2 mitotic arrest deficient-like 1 (yeast)	2.47
<i>SLC43A1</i>	solute carrier family 43, member 1	2.47
<i>SPFH1</i>	SPFH domain family, member 1	2.47
<i>ABCA1</i>	ATP-binding cassette, sub-family A (ABC1), member 1	2.47
<i>FASN</i>	fatty acid synthase	2.46
<i>RFX5</i>	regulatory factor X, 5 (influences HLA class II expression)	2.46
<i>POLR3D</i>	polymerase (RNA) III (DNA directed) polypeptide D, 44kDa	2.45
<i>SFRS6</i>	splicing factor, arginine/serine-rich 6	2.45
<i>C21orf33</i>	chromosome 21 open reading frame 33	2.45
<i>CTDSP2</i>	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 2	2.45
<i>IMPDH2</i>	IMP (inosine monophosphate) dehydrogenase 2	2.44
<i>TM4SF5</i>	transmembrane 4 L six family member 5	2.44
<i>PGCP</i>	plasma glutamate carboxypeptidase	2.44
<i>RFXANK</i>	regulatory factor X-associated ankyrin-containing protein	2.44
<i>SLC5A6</i>	solute carrier family 5 (sodium-dependent vitamin transporter), member 6	2.44
<i>DDOST</i>	dolichyl-diphosphooligosaccharide-protein glycosyltransferase	2.44
<i>FMR1</i>	fragile X mental retardation 1	2.44
<i>YIF1A</i>	Yip1 interacting factor homolog A (S. cerevisiae)	2.43
<i>ASGR2</i>	asialoglycoprotein receptor 2	2.43
<i>GGCX</i>	gamma-glutamyl carboxylase	2.43

Symbol	Gene name	Fold change
<i>JTV1</i>	JTV1 gene	2.43
<i>CPS1</i>	carbamoyl-phosphate synthetase 1, mitochondrial	2.43
<i>WDR3</i>	WD repeat domain 3	2.43
<i>HOXD1</i>	homeobox D1	2.42
<i>SYNC1</i>	syncollin, intermediate filament 1 /// syncollin, intermediate filament 1	2.42
<i>PAK3 /// UBE2C</i>	p21 (CDKN1A)-activated kinase 3 /// ubiquitin-conjugating enzyme E2C	2.42
<i>DHRS2</i>	dehydrogenase/reductase (SDR family) member 2	2.42
<i>DKC1</i>	dyskeratosis congenita 1, dyskerin	2.42
<i>FAM8A1</i>	family with sequence similarity 8, member A1	2.42
<i>SEPHS1</i>	selenophosphate synthetase 1	2.42
<i>NFATC3</i>	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3	2.41
<i>PDIA3</i>	protein disulfide isomerase family A, member 3	2.41
<i>PAPSS1</i>	3'-phosphoadenosine 5'-phosphosulfate synthase 1	2.41
<i>MERTK</i>	c-mer proto-oncogene tyrosine kinase	2.41
<i>CCL14 /// CCL15</i>	chemokine (C-C motif) ligand 14 /// chemokine (C-C motif) ligand 15	2.40
<i>IGF2 /// INS-IGF2</i>	insulin-like growth factor 2 (somatomedin A) /// insulin- insulin-like growth factor 2	2.40
<i>POLR1D</i>	polymerase (RNA) I polypeptide D, 16kDa	2.40
<i>RYK</i>	RYK receptor-like tyrosine kinase	2.40
<i>ELF2</i>	E74-like factor 2 (ets domain transcription factor)	2.40
<i>RPL5</i>	ribosomal protein L5	2.40
<i>FBXL4</i>	F-box and leucine-rich repeat protein 4	2.39
<i>POLE2</i>	polymerase (DNA directed), epsilon 2 (p59 subunit)	2.39
<i>RBBP7</i>	retinoblastoma binding protein 7	2.39
<i>AKAP1</i>	A kinase (PRKA) anchor protein 1	2.39
<i>HNRPA1</i>	heterogeneous nuclear ribonucleoprotein A1 /// heterogeneous nuclear ribonucleoprotein A1	2.39
<i>ZFR</i>	zinc finger RNA binding protein	2.39
<i>RBL2</i>	retinoblastoma-like 2 (p130)	2.38
<i>BSG</i>	basigin (Ok blood group)	2.38
<i>FXYD2 /// MB</i>	FXYD domain containing ion transport regulator 2 /// myoglobin	2.38
<i>RBKS</i>	ribokinase	2.38
<i>IL1R1</i>	interleukin 1 receptor, type I	2.38
<i>EEF1B2</i>	eukaryotic translation elongation factor 1 beta 2	2.38
<i>GDI2</i>	GDP dissociation inhibitor 2 /// GDP dissociation inhibitor 2	2.37
<i>EIF2S3</i>	eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa	2.37
<i>ISYNA1</i>	myo-inositol 1-phosphate synthase A1	2.37
<i>LSM4</i>	LSM4 homolog, U6 small nuclear RNA associated (S. cerevisiae)	2.37
<i>SFPQ</i>	splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated)	2.37
<i>AMBP</i>	alpha-1-microglobulin/bikunin precursor	2.37
<i>USP1</i>	ubiquitin specific peptidase 1	2.36
<i>CEBPD</i>	CCAAT/enhancer binding protein (C/EBP), delta	2.36
<i>GNL3</i>	guanine nucleotide binding protein-like 3 (nucleolar)	2.35
<i>FBLN1</i>	fibulin 1	2.35
<i>CANX</i>	calnexin /// calnexin	2.35
<i>DPAGT1</i>	dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase)	2.34
<i>ZNF124</i>	zinc finger protein 124	2.34
<i>USP18 /// LOC727996 ///</i>	ubiquitin specific peptidase 18 /// similar to ubiquitin specific peptidase 18 /// similar to ubiquitin specific peptidase 18 /// similar to ubiquitin specific peptidase 18	2.34

Symbol	Gene name	Fold change
<i>LOC728216</i> /// <i>LOC728438</i>		
<i>GCS1</i>	glucosidase I	2.34
<i>AUH</i>	AU RNA binding protein/enoyl-Coenzyme A hydratase	2.34
<i>RBM8A</i>	RNA binding motif protein 8A	2.33
<i>SRPRB</i>	signal recognition particle receptor, B subunit	2.33
<i>TLE1</i>	transducin-like enhancer of split 1 (E(sp1) homolog, Drosophila)	2.32
<i>VPS28</i>	vacuolar protein sorting 28 homolog (S. cerevisiae)	2.32
<i>MAGEF1</i>	melanoma antigen family F, 1	2.31
<i>ACVR2B</i>	activin A receptor, type IIB	2.31
<i>DLK1</i>	delta-like 1 homolog (Drosophila)	2.31
<i>SMO</i>	smoothened homolog (Drosophila)	2.31
<i>ACAA1</i>	acetyl-Coenzyme A acyltransferase 1 (peroxisomal 3-oxoacyl-Coenzyme A thiolase)	2.30
<i>SC65</i>	synaptonemal complex protein SC65	2.30
<i>CRTAP</i>	cartilage associated protein	2.29
<i>TWF2</i>	twinfilin, actin-binding protein, homolog 2 (Drosophila)	2.29
<i>DHFR</i>	dihydrofolate reductase	2.29
<i>LAMC1</i>	laminin, gamma 1 (formerly LAMB2)	2.28
<i>PPP1CC</i>	protein phosphatase 1, catalytic subunit, gamma isoform	2.28
<i>FXYD3</i>	FXYD domain containing ion transport regulator 3	2.27
<i>C6orf108</i>	chromosome 6 open reading frame 108	2.27
<i>DNAJA3</i>	DnaJ (Hsp40) homolog, subfamily A, member 3	2.26
<i>MBOAT5</i>	membrane bound O-acyltransferase domain containing 5	2.26
<i>LMAN2</i>	lectin, mannose-binding 2	2.26
<i>MAD1L1</i>	MAD1 mitotic arrest deficient-like 1 (yeast)	2.26
<i>SNRPN</i> /// <i>SNURF</i>	small nuclear ribonucleoprotein polypeptide N /// SNRPN upstream reading frame	2.26
		2.25
<i>SDCCAG3</i>	serologically defined colon cancer antigen 3	2.25
<i>SLC39A7</i>	solute carrier family 39 (zinc transporter), member 7	2.25
<i>ATP5G2</i>	ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit C2 (subunit 9)	2.24
<i>MCM6</i>	minichromosome maintenance deficient 6 homolog (S. cerevisiae)	2.24
<i>LAPTM4A</i>	lysosomal-associated protein transmembrane 4 alpha	2.24
<i>GDI2</i>	GDP dissociation inhibitor 2 /// GDP dissociation inhibitor 2	2.23
<i>METTL1</i>	methyltransferase like 1	2.23
<i>SELENBP1</i>	selenium binding protein 1 /// selenium binding protein 1	2.23
<i>CYP27A1</i>	cytochrome P450, family 27, subfamily A, polypeptide 1	2.23
<i>SLC11A2</i>	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 2	2.23
<i>FKBP3</i>	FK506 binding protein 3, 25kDa	2.23
<i>IFNGR2</i>	interferon gamma receptor 2 (interferon gamma transducer 1)	2.22
<i>SLC4A7</i>	solute carrier family 4, sodium bicarbonate cotransporter, member 7	2.22
<i>MTMR4</i>	myotubularin related protein 4	2.22
<i>SLC31A1</i>	solute carrier family 31 (copper transporters), member 1	2.22
<i>ALDH1L1</i>	aldehyde dehydrogenase 1 family, member L1	2.21
<i>GMPR2</i>	guanosine monophosphate reductase 2	2.21
<i>RGNEF</i>	Rho-guanine nucleotide exchange factor	2.21
<i>APOC1</i>	apolipoprotein C-I	2.21
<i>CDC14B</i>	CDC14 cell division cycle 14 homolog B (S. cerevisiae) /// CDC14 cell division cycle 14 homolog B (S. cerevisiae)	2.21

Symbol	Gene name	Fold change
<i>CHES1</i>	checkpoint suppressor 1	2.21
<i>PPAN</i>	peter pan homolog (Drosophila)	2.21
<i>TMEM165</i>	transmembrane protein 165	2.20
<i>CHPT1</i>	choline phosphotransferase 1	2.20
<i>RPS6KA3</i>	ribosomal protein S6 kinase, 90kDa, polypeptide 3	2.20
<i>CANX</i>	calnexin	2.20
<i>SIRT3</i>	sirtuin (silent mating type information regulation 2 homolog) 3 (S. cerevisiae)	2.20
<i>PUM2</i>	pumilio homolog 2 (Drosophila)	2.19
<i>FAM60A</i>	family with sequence similarity 60, member A	2.19
<i>SEMA3C</i>	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	2.19
<i>TNFSF4</i>	tumor necrosis factor (ligand) superfamily, member 4 (tax-transcriptionally activated glycoprotein 1, 34kDa)	2.19
<i>DDX17</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	2.19
<i>CEPT1</i>	choline/ethanolamine phosphotransferase 1	2.18
<i>CCNA2</i>	cyclin A2	2.18
<i>TRAM1</i>	translocation associated membrane protein 1	2.18
<i>CTGF</i>	connective tissue growth factor	2.18
<i>FH</i>	fumarate hydratase	2.18
<i>PRCP</i>	prolylcarboxypeptidase (angiotensinase C)	2.17
<i>SFRS10</i>	splicing factor, arginine/serine-rich 10 (transformer 2 homolog, Drosophila)	2.17
<i>SLC25A5</i>	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5	2.16
<i>RABL2B</i> /// <i>RABL2A</i>	RAB, member of RAS oncogene family-like 2B /// RAB, member of RAS oncogene family-like 2A	2.16
<i>ANAPC5</i>	anaphase promoting complex subunit 5	2.16
<i>GLTSCR2</i>	glioma tumor suppressor candidate region gene 2	2.16
<i>LSM6</i> /// <i>CHD9</i>	LSM6 homolog, U6 small nuclear RNA associated (S. cerevisiae) /// chromodomain helicase DNA binding protein 9	2.15
<i>CHD1L</i>	chromodomain helicase DNA binding protein 1-like	2.15
<i>AKR1A1</i>	aldo-keto reductase family 1, member A1 (aldehyde reductase)	2.15
<i>RPLP0</i> /// <i>RPLP0-like</i>	ribosomal protein, large, P0 /// similar to ribosomal protein P0	2.15
<i>SMARCA1</i>	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 1	2.14
<i>PPOX</i>	protoporphyrinogen oxidase	2.14
<i>SLC22A3</i>	solute carrier family 22 (extraneuronal monoamine transporter), member 3	2.14
<i>PRKAB1</i>	protein kinase, AMP-activated, beta 1 non-catalytic subunit	2.14
<i>SNRPN</i> /// <i>SNURF</i>	small nuclear ribonucleoprotein polypeptide N /// SNRPN upstream reading frame	2.13
<i>HYAL2</i>	hyaluronoglucosaminidase 2	2.13
<i>PSMB9</i>	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	2.13
<i>CDK5RAP3</i>	CDK5 regulatory subunit associated protein 3	2.13
<i>RCN2</i>	reticulocalbin 2, EF-hand calcium binding domain	2.13
<i>SFRS1</i>	splicing factor, arginine/serine-rich 1 (splicing factor 2, alternate splicing factor)	2.13
<i>RFC3</i>	replication factor C (activator 1) 3, 38kDa	2.13
<i>BAT1</i>	HLA-B associated transcript 1 /// HLA-B associated transcript 1	2.12
<i>MTR</i>	5-methyltetrahydrofolate-homocysteine methyltransferase	2.12
<i>AFG3L2</i>	AFG3 ATPase family gene 3-like 2 (yeast)	2.12
<i>RPN1</i>	ribophorin I	2.12

Symbol	Gene name	Fold change
<i>PKP2</i>	plakophilin 2	2.12
<i>PCYT2</i>	phosphate cytidyltransferase 2, ethanolamine	2.11
<i>DGCR2</i>	DiGeorge syndrome critical region gene 2	2.11
<i>RUVBL1</i>	RuvB-like 1 (E. coli)	2.11
<i>ANP32A</i>	acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	2.11
<i>ENDOG</i>	endonuclease G	2.11
<i>HAMP</i>	hepcidin antimicrobial peptide	2.11
<i>ERP29</i>	endoplasmic reticulum protein 29	2.11
<i>PDE3B</i>	phosphodiesterase 3B, cGMP-inhibited	2.10
<i>SLC35E3</i>	solute carrier family 35, member E3	2.10
<i>MSH2</i>	mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)	2.10
<i>UGCGL2</i>	UDP-glucose ceramide glucosyltransferase-like 2	2.10
<i>E2F1</i>	E2F transcription factor 1	2.09
<i>TIAL1</i>	TIA1 cytotoxic granule-associated RNA binding protein-like 1	2.09
<i>GSTM4</i>	glutathione S-transferase M4	2.09
<i>DIAPH2</i>	diaphanous homolog 2 (Drosophila)	2.09
<i>NME4</i>	non-metastatic cells 4, protein expressed in	2.09
<i>ACOX2</i>	acyl-Coenzyme A oxidase 2, branched chain	2.09
<i>UTP14A</i>	UTP14, U3 small nucleolar ribonucleoprotein, homolog A (yeast)	2.08
<i>ETHE1</i>	ethylmalonic encephalopathy 1	2.08
<i>INVS</i>	inversin	2.08
<i>DUSP6</i>	dual specificity phosphatase 6	2.08
<i>ADSL</i>	adenylosuccinate lyase	2.08
<i>DVL2</i>	dishevelled, dsh homolog 2 (Drosophila)	2.07
<i>SERGEF</i>	secretion regulating guanine nucleotide exchange factor	2.07
<i>PCM1 /// TPTE</i>	pericentriolar material 1 /// transmembrane phosphatase with tensin homology	2.07
<i>EPPB9</i>	B9 protein	2.06
<i>CDKN1B</i>	cyclin-dependent kinase inhibitor 1B (p27, Kip1)	2.06
<i>ZNF236</i>	zinc finger protein 236	2.06
<i>ICAM1</i>	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	2.06
<i>PLSCR1</i>	phospholipid scramblase 1	2.06
<i>ALB</i>	albumin	2.05
<i>MTM1</i>	myotubularin 1	2.05
<i>DBI</i>	diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein)	2.05
<i>DDX21</i>	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21 /// DEAD (Asp-Glu-Ala-Asp) box polypeptide 21	2.05
<i>SLC25A20</i>	solute carrier family 25 (carnitine/acylcarnitine translocase), member 20	2.05
<i>SSR1</i>	signal sequence receptor, alpha (translocon-associated protein alpha)	2.05
<i>WDR42A</i>	WD repeat domain 42A	2.05
<i>SEC13</i>	SEC13 homolog (S. cerevisiae)	2.04
<i>SEC31A</i>	SEC31 homolog A (S. cerevisiae)	2.04
<i>TMED3</i>	transmembrane emp24 protein transport domain containing 3	2.04
<i>POLA1</i>	polymerase (DNA directed), alpha 1	2.04
<i>RAD50</i>	RAD50 homolog (S. cerevisiae)	2.04
<i>NPM1</i>	nucleophosmin (nucleolar phosphoprotein B23, numatrin) /// nucleophosmin (nucleolar phosphoprotein B23, numatrin)	2.03
<i>MUTYH</i>	mutY homolog (E. coli)	2.03
	CDNA FLJ40810 fis, clone TRACH2009743	2.03

Symbol	Gene name	Fold change
<i>TYMS</i>	thymidylate synthetase	2.03
<i>TMEM4</i>	transmembrane protein 4	2.03
<i>BCKDHB</i>	branched chain keto acid dehydrogenase E1, beta polypeptide (maple syrup urine disease)	2.02
<i>FNTB</i>	farnesyltransferase, CAAX box, beta	2.02
<i>BLMH</i>	bleomycin hydrolase	2.02
<i>NANS</i>	N-acetylneuraminic acid synthase (sialic acid synthase)	2.02
<i>RPL29</i>	ribosomal protein L29	2.02
<i>PMPCB</i>	peptidase (mitochondrial processing) beta	2.01
<i>XPOT</i>	exportin, tRNA (nuclear export receptor for tRNAs)	2.01
<i>FUSIP1 /// LOC727922</i>	FUS interacting protein (serine/arginine-rich) 1 /// similar to FUS-interacting serine-arginine-rich protein 1 (TLS-associated protein with Ser-Arg repeats) (TLS-associated protein with SR repeats) (TASR) (TLS-associated serine-arginine protein) (TLS-associated SR protein) (Neural-specific SR protein...	2.01
<i>PPAT</i>	phosphoribosyl pyrophosphate amidotransferase	2.01
<i>WASF3</i>	WAS protein family, member 3	2.00
<i>EIF3S6</i>	eukaryotic translation initiation factor 3, subunit 6 48kDa	2.00
<i>ADCY9</i>	adenylate cyclase 9	2.00
<i>FUSIP1 /// LOC727922</i>	FUS interacting protein (serine/arginine-rich) 1 /// similar to FUS-interacting serine-arginine-rich protein 1 (TLS-associated protein with Ser-Arg repeats) (TLS-associated protein with SR repeats) (TASR) (TLS-associated serine-arginine protein) (TLS-associated SR protein) (Neural-specific SR protein...	2.00
<i>TFAM</i>	transcription factor A, mitochondrial	2.00

6 a) Up-regulated genes in Caprolactam exposure

Symbol	Gene name	Fold change
<i>AQP3</i>	aquaporin 3 (Gill blood group)	9.36
<i>DUSP5</i>	dual specificity phosphatase 5	6.07
<i>PHLDA2</i>	pleckstrin homology-like domain, family A, member 2	5.72
<i>TGFB1</i>	transforming growth factor, beta 1 (Camurati-Engelmann disease)	4.31
<i>CD55</i>	CD55 molecule, decay accelerating factor for complement (Cromer blood group)	3.97
<i>LGALS3</i>	lectin, galactoside-binding, soluble, 3 (galectin 3)	3.91
<i>EGR1</i>	early growth response 1	3.39
<i>PTPRH</i>	protein tyrosine phosphatase, receptor type, H	3.18
<i>COTL1</i>	coactosin-like 1 (Dictyostelium)	3.06
<i>MAP2K2</i>	mitogen-activated protein kinase kinase 2	3.03
<i>UPP1</i>	uridine phosphorylase 1	3.00
<i>UBL3</i>	ubiquitin-like 3	2.86
<i>CDC42EP1</i>	CDC42 effector protein (Rho GTPase binding) 1	2.84
<i>TIMP1</i>	TIMP metalloproteinase inhibitor 1	2.82
<i>FDX1</i>	ferredoxin 1	2.82
<i>AKAP12</i>	A kinase (PRKA) anchor protein (gravin) 12	2.75
<i>SRC</i>	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	2.69
<i>HYOU1</i>	hypoxia up-regulated 1	2.65
	CDNA FLJ34214 fis, clone FCBBF3021807	2.58
<i>SFRS7</i>	splicing factor, arginine/serine-rich 7, 35kDa	2.54
<i>ACSL3</i>	acyl-CoA synthetase long-chain family member 3	2.53
<i>CBX4</i>	chromobox homolog 4 (Pc class homolog, Drosophila)	2.51
<i>ZYX</i>	zyxin	2.48
<i>RPL31</i>	ribosomal protein L31	2.48
<i>GPCAL1</i>	hippocalcin-like 1	2.48
<i>GCLC</i>	glutamate-cysteine ligase, catalytic subunit	2.46
<i>DNAJB9</i>	DnaJ (Hsp40) homolog, subfamily B, member 9	2.46
<i>ASPH</i>	aspartate beta-hydroxylase	2.44
<i>F2RL1</i>	coagulation factor II (thrombin) receptor-like 1	2.44
<i>SEC23B</i>	Sec23 homolog B (S. cerevisiae)	2.43
<i>CALM1</i>	calmodulin 1 (phosphorylase kinase, delta)	2.43
<i>KCNMB3</i>	potassium large conductance calcium-activated channel, subfamily M beta member 3	2.39
<i>IER2</i>	immediate early response 2	2.37
<i>ANKRD11</i>	ankyrin repeat domain 11	2.36
<i>MAP1LC3B</i>	microtubule-associated protein 1 light chain 3 beta	2.33
<i>CSTA</i>	cystatin A (stefin A)	2.31
<i>NKX3-1</i>	NK3 transcription factor related, locus 1 (Drosophila)	2.30
<i>LOC388796</i>	hypothetical LOC388796	2.29
<i>PPP1R15A</i>	protein phosphatase 1, regulatory (inhibitor) subunit 15A	2.27
<i>TXNIP</i>	thioredoxin interacting protein	2.27
<i>SERPINE1</i>	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	2.26
<i>JAG1</i>	jagged 1 (Alagille syndrome)	2.22
<i>ARMET</i>	arginine-rich, mutated in early stage tumors	2.22
<i>HIST1H2AC</i>	histone cluster 1, H2ac	2.20
<i>FABP5</i> /// <i>LOC728641</i> /// <i>LOC729163</i> ///	fatty acid binding protein 5 (psoriasis-associated) /// similar to Fatty acid-binding protein, epidermal (E-FABP) (Psoriasis-associated fatty acid-binding protein homolog) (PA-FABP) /// similar to Fatty acid-binding protein, epidermal (E-FABP)	2.20

Symbol	Gene name	Fold change
<i>LOC731043</i> /// <i>LOC732031</i>	(Psoriasis-associated fatty acid-binding protein homolog) (PA-FABP) /// similar to Fatty acid-binding protein, epidermal (E-FABP) (Psoriasis-associated fatty acid-binding protein homolog) (PA-FABP) /// similar to Fatty acid-binding protein, epidermal (E-FABP) (Psoriasis-associated fatty acid-binding protein homolog) (PA-FABP)	
<i>MYO6</i>	myosin VI	2.20
<i>SIP1</i>	survival of motor neuron protein interacting protein 1	2.20
<i>PPARGC1A</i>	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	2.18
<i>PCK1</i>	phosphoenolpyruvate carboxykinase 1 (soluble)	2.18
<i>VPS4A</i>	vacuolar protein sorting 4 homolog A (S. cerevisiae)	2.17
<i>NPC1L1</i>	NPC1 (Niemann-Pick disease, type C1, gene)-like 1	2.17
<i>LRP8</i>	low density lipoprotein receptor-related protein 8, apolipoprotein e receptor	2.17
<i>MCL1</i>	myeloid cell leukemia sequence 1 (BCL2-related)	2.16
<i>PNMA1</i>	paraneoplastic antigen MA1	2.16
<i>ATF5</i>	activating transcription factor 5	2.15
<i>LOC399491</i>	LOC399491 protein	2.14
<i>ST6GALNAC4</i>	ST6 (alpha-N-acetyl-neuraminy-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 4	2.14
<i>HSPA1A</i> /// <i>HSPA1B</i>	heat shock 70kDa protein 1A /// heat shock 70kDa protein 1B	2.13
<i>IGSF1</i>	immunoglobulin superfamily, member 1	2.12
<i>PPAP2A</i>	phosphatidic acid phosphatase type 2A	2.12
<i>TNFRSF12A</i>	tumor necrosis factor receptor superfamily, member 12A	2.12
<i>EIF4G3</i>	eukaryotic translation initiation factor 4 gamma, 3	2.12
<i>TESK1</i>	testis-specific kinase 1	2.11
<i>WNK1</i>	WNK lysine deficient protein kinase 1 /// WNK lysine deficient protein kinase 1	2.10
<i>ADORA2B</i>	adenosine A2b receptor	2.10
<i>HSPA1B</i>	heat shock 70kDa protein 1B	2.10
		2.09
<i>C1orf9</i>	chromosome 1 open reading frame 9	2.08
<i>TSC2</i>	tuberous sclerosis 2	2.07
<i>LDLR</i>	low density lipoprotein receptor (familial hypercholesterolemia)	2.05
<i>CHUK</i>	conserved helix-loop-helix ubiquitous kinase	2.04
<i>ARHGEF2</i>	rho/rac guanine nucleotide exchange factor (GEF) 2	2.04
<i>H1FX</i>	H1 histone family, member X	2.03
<i>MKNK2</i>	MAP kinase interacting serine/threonine kinase 2	2.02
<i>FGFR1</i>	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	2.02
<i>MAP2K3</i>	mitogen-activated protein kinase kinase 3 /// mitogen-activated protein kinase kinase 3	2.02
<i>DUSP6</i>	dual specificity phosphatase 6	2.00
<i>RNF141</i>	ring finger protein 141	2.00

6 b) Down-regulated genes in Caprolactam exposure

Symbol	Gene name	Fold change
<i>MBL2</i>	mannose-binding lectin (protein C) 2, soluble (opsonic defect)	11.37
<i>DKK1</i>	dickkopf homolog 1 (<i>Xenopus laevis</i>)	6.82
<i>PGC</i>	progastricsin (pepsinogen C)	6.60
<i>TUBA3</i>	tubulin, alpha 3	5.26
<i>UBD</i>	ubiquitin D	5.17
<i>MATN3</i>	matrilin 3	5.16
<i>DDB2</i>	damage-specific DNA binding protein 2, 48kDa	5.12
<i>CD9</i>	CD9 molecule	5.10
<i>SOX4</i>	SRY (sex determining region Y)-box 4	3.99
<i>MCM5</i>	MCM5 minichromosome maintenance deficient 5, cell division cycle 46 (<i>S. cerevisiae</i>)	3.95
<i>HOXD1</i>	homeobox D1	3.88
<i>GIN52</i>	GIN5 complex subunit 2 (Psf2 homolog)	3.49
<i>LGALS2</i>	lectin, galactoside-binding, soluble, 2 (galectin 2) /// lectin, galactoside-binding, soluble, 2 (galectin 2)	3.48
<i>KDEL3</i>	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3	3.32
<i>CPB2</i>	carboxypeptidase B2 (plasma, carboxypeptidase U)	3.31
<i>BAX</i>	BCL2-associated X protein	3.19
<i>HSPB1</i> /// <i>MEIS3</i>	heat shock 27kDa protein 1 /// Meis1, myeloid ecotropic viral integration site 1 homolog 3 (mouse)	3.19
<i>SLC6A14</i>	solute carrier family 6 (amino acid transporter), member 14	3.11
<i>GPX2</i>	glutathione peroxidase 2 (gastrointestinal)	3.02
<i>MCM2</i>	MCM2 minichromosome maintenance deficient 2, mitotin (<i>S. cerevisiae</i>)	3.02
<i>PDZK1</i>	PDZ domain containing 1	3.00
<i>SLC04A1</i>	solute carrier organic anion transporter family, member 4A1	2.97
<i>NQO1</i>	NAD(P)H dehydrogenase, quinone 1	2.91
<i>C4BPA</i>	complement component 4 binding protein, alpha	2.80
<i>PRIM1</i>	primase, polypeptide 1, 49kDa	2.79
<i>FGB</i>	fibrinogen beta chain	2.77
<i>IDH2</i>	isocitrate dehydrogenase 2 (NADP+), mitochondrial	2.68
<i>RPA3</i>	replication protein A3, 14kDa	2.65
<i>FRY</i>	furry homolog (<i>Drosophila</i>)	2.64
<i>SPP1</i>	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	2.59
<i>SERPINA7</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7	2.59
<i>FXDY2</i> /// <i>MB</i>	FXDY domain containing ion transport regulator 2 /// myoglobin	2.54
<i>VAV3</i>	vav 3 oncogene	2.54
<i>CP</i>	ceruloplasmin (ferroxidase)	2.48
<i>POLE2</i>	polymerase (DNA directed), epsilon 2 (p59 subunit)	2.48
<i>MAGED1</i>	melanoma antigen family D, 1	2.44
<i>LGR5</i>	leucine-rich repeat-containing G protein-coupled receptor 5	2.41
<i>DIO1</i>	deiodinase, iodothyronine, type I	2.40
<i>MCM3</i>	MCM3 minichromosome maintenance deficient 3 (<i>S. cerevisiae</i>)	2.39
<i>BTN3A3</i> /// <i>BTN3A2</i>	butyrophilin, subfamily 3, member A3 /// butyrophilin, subfamily 3, member A2	2.38
<i>POLA1</i>	polymerase (DNA directed), alpha 1	2.37
<i>CFI</i>	complement factor I	2.37
<i>HIST1H4C</i>	histone cluster 1, H4c	2.35
<i>COCH</i>	coagulation factor C homolog, cochlin (<i>Limulus polyphemus</i>)	2.32

Symbol	Gene name	Fold change
<i>PAPSS1</i>	3'-phosphoadenosine 5'-phosphosulfate synthase 1	2.31
<i>RNASEH2A</i>	ribonuclease H2, subunit A	2.30
<i>PLEKHB1</i>	pleckstrin homology domain containing, family B (evectins) member 1	2.30
<i>PCNA</i>	proliferating cell nuclear antigen	2.29
<i>PAM</i>	peptidylglycine alpha-amidating monooxygenase	2.28
<i>POU2AF1</i>	POU domain, class 2, associating factor 1	2.25
<i>TAGLN</i>	transgelin	2.23
<i>CAT</i>	catalase	2.21
<i>VAMP8</i>	vesicle-associated membrane protein 8 (endobrevin)	2.21
<i>SORBS1</i>	sorbin and SH3 domain containing 1	2.20
<i>RFC4</i>	replication factor C (activator 1) 4, 37kDa	2.19
<i>B3GNT1</i>	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1	2.15
<i>ID3</i>	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	2.14
<i>CASP6</i>	caspase 6, apoptosis-related cysteine peptidase	2.14
<i>RFC1</i>	replication factor C (activator 1) 1, 145kDa /// replication factor C (activator 1) 1, 145kDa	2.12
<i>MADCAM1</i>	mucosal vascular addressin cell adhesion molecule 1	2.12
<i>RGNEF</i>	Rho-guanine nucleotide exchange factor	2.11
<i>RRM2</i>	ribonucleotide reductase M2 polypeptide	2.10
<i>CALR</i>	Calreticulin	2.10
<i>ADH6</i>	alcohol dehydrogenase 6 (class V)	2.09
<i>SPAG4</i>	sperm associated antigen 4	2.08
<i>ZWINT</i>	ZW10 interactor	2.07
<i>FAS</i>	Fas (TNF receptor superfamily, member 6)	2.07
<i>P4HA2</i>	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide II	2.07
<i>TNFAIP3</i>	tumor necrosis factor, alpha-induced protein 3	2.06
<i>CEACAM1</i>	carcinoembryonic antigen-related cell adhesion molecule 1 (biliary glycoprotein)	2.06
<i>FADS2</i>	fatty acid desaturase 2	2.05
<i>SERPINA3</i>	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	2.04
<i>RAB17</i>	RAB17, member RAS oncogene family	2.04
<i>PLS3</i>	plastin 3 (T isoform)	2.03
<i>P2RY5</i>	purinergic receptor P2Y, G-protein coupled, 5	2.03
<i>SGK</i>	serum/glucocorticoid regulated kinase	2.02
<i>SGK2</i>	serum/glucocorticoid regulated kinase 2	2.01
<i>MSH2</i>	mutS homolog 2, colon cancer, nonpolyposis type 1 (<i>E. coli</i>)	2.00
<i>PROM1</i>	prominin 1	2.00
<i>RAPGEF5</i>	Rap guanine nucleotide exchange factor (GEF) 5	2.00
<i>HMGA2</i>	high mobility group AT-hook 2 /// high mobility group AT-hook 2	2.00