Proteomic and Genomic Analysis of Interleukin-1β-Treated NCI-H292 Cells

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Proteomic and Genomic Analysis of Interleukin-1β-Treated NCI-H292 Cells

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This certifies that the Doctoral Dissertation of Janghoon Chi is approved.

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Yonsei University

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또한 연구기간 중 아낌없는 도움을 주신 김창훈 교수님을 비롯하여, 이비인후과학교실의 교수님과 의국원들께 진심으로 감사를 드립니다.

끝으로 항상 곁에서 따뜻하게 격려하여 주신 부모님과 가족 모두에게도 감사의 마음을 전합니다.

저 자 씀

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List of Abbreviations

IL: Interleukin

MUC: mucin

IEF: isoelectric focusing

2-D PAGE: 2-dimensional polyacrylamide gel electrophoresis

2-DE: 2-dimensional electrophoresis

COPD: chronic obstructive pulmonary disease

MALDI-TOF: matrix-assisted laser desorption/ionization – time of flight

MS: mass spectrometry

IPG: immobilized pH gradient

ACN: acetonitrile

ABC: ammonium bicarbonate

CHCA: α-cyano-4-hydroxycinnamic acid

HSP: heat shock protein

SLPI: secretory leukocyte protease inhibitor

Abstract

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Mucin hypersecretion is one of main pathogenesis causing inflammatory disease in respiratory tract. We showed that the pleiotypic proinflammatory cytokine, interleukin (IL)-1β, could play significant roles in respiratory tract inflammation by the induction of mucins (MUC2, MUC5AC, MUC8). However, it still remains unclear about the molecular mechanism to induce mucin hypersecretion in respiratory tract. To understand the mechanisms of mucin hypersecretion in airway epithelium, we identified differentially expressed proteins and genes in lung mucoepidermoid carcinoma cell line (NCI-H292 cells) on 6 and 24 hrs after treatment of IL-1β (10 ng/ml) by 2-dimensional polyacrylamide gel electrophoresis (2-D PAGE) proteomics and cDNA microarray analysis (8.6K). In the 2-D PAGE, we identified 8 differentially expressed proteins and 14 post-translational modification proteins on 6 and 24 hrs after treatment of IL-1β. A total 413 genes (6.6%) and 115 genes (2.0%) were found to be regulated after IL-1β treatment, respectively. Differentially expressed genes regulated by IL-1β treatment were mostly in metabolic pathway rather than regulatory pathway. By comparison of proteomic and microarray data, there was large discrepancy between gene expression and protein expression levels.

Among the genes encode secreted protein of airway, MUC5B is down-regulated but sialomucin CD 164, lysozyme, and secretory leukocyte protease inhibitor (SLPI) are up-regulated. Our results clearly indicate that transcript levels provide little predictive value with the extent of protein expression. Due to the quite different scopes of evaluating fields between genomics and proteomics, they may not provide complete information on the gene and protein profiles.

Key words : 2-dimensional polyacrylamide gel electrophoresis, cDNA microarray, mucus, hypersecretion

Proteomic and Genomic Analysis of Interleukin-1β-Treated NCI-H292 Cells

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I. INTRODUCTION

Airway epithelium physiologically secrets mucus. However oversecreted mucus can produce the most troublesome symptoms in most inflammatory airway diseases, such as rhinitis, sinusitis, asthma and chronic bronchitis.[1-4] Mucins are major component of the mucus but so many other proteins from airway epithelium comprise airway mucus. But there has been few profile of protein from airway epithelium and those functional roles of proteins are not known. We understand that these proteins can give us complete information of airway epithelium and patterns of these proteins would be a mirror of biological behavior in airway epithelium. Furthermore altered profile of protein and gene can give us an open window for various airway diseases.

Interleukin (IL)-1β play a critical role in a variety of airway inflammatory diseases including rhinitis, sinusitis, asthma, chronic obstructive pulmonary disease (COPD), chronic bronchitis, cystic fibrosis, and emphysema.[5] IL-1β has been also known to induce mucins (MUC2, MUC5AC, MUC8) that have important role in pathophysiology of inflammatory lung disease.[6,7] However, profile of genes and

proteins related to induce hypersecretion of mucin affected by IL-1β in airway epithelium has not yet been fully explored.

We analyzed the effect of the IL-1β on differentially expressed gene and protein profile related to hypersecretion of mucin affected by IL-1β from lung mucoepidermoid carcinoma cell line (NCI-H292 cells) using 2-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and Matrix-assisted laser desorption/ionization – time of flight (MALDI-TOF) mass spectrometry (MS) and cDNA microarray.

II. MATERIALS AND METHODS

1. Cell culture and treatment

The human lung mucoepidermoid carcinoma cell line (NCI-H292), was purchased from the American Type Culture Collection (CRL-1848; Manassas, VA, USA) and cultured in RPMI-1640 (GIBCO BRL, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS) in the presence of penicillin-streptomycin at 37°C in a humidified chamber with 5% CO_2. Two days after confluence, 10 ng/ml of IL-1β was treated for 0, 6, and 24 hrs and media was discarded. Cell surface was washed with sterile phosphate-buffered saline (PBS) twice, and samples were harvested.

2. Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE)

A. Sample preparation

The cells were removed immediately and stored at –80°C after freezing in liquid nitrogen prior to preparation for 2-DE. The samples were collected and homogenized in 400 μl lysis buffer (7M urea, 2M thiourea, 2% CHAPS, 2% Pharmalyte pH 3-10 [Pharmacia Corporation, Peapack, NJ, USA], 100 mM DTE) and protease inhibitor (complete protease inhibitor cocktail tablets, Roche Applied Science, Indianapolis, IN, USA) was added. To remove solid parts, the samples were centrifuged at 12,000 x *g* at 4°C for 1 hr. The supernatant was transferred into new microcentrifuge tube. Solubilized protein samples were analyzed quantitatively by Bradford methods, and divided into 1 mg protein per aliquots and stored at –80°C.

B. 2-D PAGE

The proteins were diluted in lysis buffer to make final 450 μl. The samples were applied to the 240 mm, immobilized pH gradient (IPG) strips of pH 3-10, nonlinear (IPG Drystrips, Amersham Biosciences, Piscataway, NJ, USA), which was rehydrated for at least 10 hrs. After rehydration, the strips were focused at 100V for 1 hr, 300V

for 1 hr, 600V for 1 hr, 1,000 V for 1 hr, 2,000V for 1 hr and finally at 3,500V for 22 hrs so as to obtain approximately 75,000 VHr (Multiphore, Amersham Biosciences., Piscataway, NJ, USA). Once IEF was completed, the strips were equilibrated in 6M urea containing 20% glycerol, 2% SDS and 0.01% bromophenol blue (BPB) with 10 mM tributyl phosphine (TBP). SDS-PAGE was performed using 10-18% T, gradient gel without stacking gel using the EttanDalt system (Hoefer, San Francisco, CA, USA). Two-dimensional electrophoresis was carried out overnight with 2 W/gel at 20°C. The gels were stained with Coomassie Blue G-250 (Bio-rad, Hercules, CA, USA).

C. Protein visualization and software analysis

The stained gels were scanned using GS-800 Calibrated Densimeter (Bio-rad, Hercules, CA, USA) and analyzed with the Mellanie III (SIB, Sweden). Digitized images were compared by matching method. Differentially expressed spots were analyzed (greater than 3-fold increase and more than 0.33-fold decrease in intensity).

D. Sample preparation for mass spectrometry

The gel pieces were washed in water for 1 hr, then in 40% methanol, 100% acetonitrile (ACN), 50 mM ammonium bicarbonate (ABC), pH 8.0 (1-3 times, approximately for 10 min each) in pre-siliconized Eppendorf tubes (PGC Scientific, Frederick, MD, USA) and finally dehydrated in a vacuum evaporator (approximately 15 min). The gel pieces were reswollen with a trypsin solution containing 0.1-0.2 μg Promega modified trypsin in 50 mM ABC, pH 8.0. Additional ABC buffer was added until the gel pieces had recovered their original size. The gels were incubated at 37°C overnight. Prior to MALDI-TOF MS analysis, the tryptic peptides were concentrated by POROS R2 column (Applied Biosystems, Foster city, CA, USA). After subsequent washing steps of column with 5% formic acid and 100% ACN, the sample was loaded into the R2 column and was eluted in 2 μl a-cyano-4-hydroxycinnamic

acid (CHCA) and then was dropped onto MALDI plate (96 x 2, Applied Biosystems, Foster city, CA, USA).

E. Mass spectrometry

MALDI-TOF MS was performed using the Applied Biosystems Voyager DE-PRO spectrometer (Applied Biosystems, Forste city, USA), equipped with a 337 nm nitrogen laser. The instrument was operated in accelerating voltage 20 kV, positive ion reflection mode, voltage grid 74.5%, guide wire voltage 0%, delay-time 120 ns. Spectra were internally calibrated using trypsin autolysis products (842.51 Da and 2211.11 Da), and the resulting peptide masses were searched database managed by the SWISS-PROT (http://kr.expasy.org/) and National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). All searches were used in 50 ppm mass tolerance.

3. cDNA microarray

Labeled cDNA were prepared using 50 μg total RNA. Total RNA was extracted from control and from IL-1β treated NCI-H292 cells using Trizol reagent (MRC, Cincinnati, OH, USA) and was added to 2 μℓ of oligo dT (18mer) primer (1 μg/μℓ). The mixture was denatured at 70°C for 10 min and then chilled on ice for 2min. Reverse transcription was performed by adding the following components to the annealed probe/template on ice: 6 μℓ of First Strand Buffer 5× (250 mM Tris-HCl pH 8.3, 375 mM KCl, 15 mM $MgCl_2$) (Invitrogen life technologies, Carlsbad, CA, USA), 3 μℓ of DTT 0.1M (Invitrogen life technologies, Carlsbad, CA, USA), 100 μM dATP(Amersham Biosciences, Piscataway, NJ, USA), 100 μM dGTP(Amersham Biosciences, Piscataway, NJ, USA), 100 μM dTTP(Amersham Biosciences, Piscataway, NJ, USA), 100 μM dCTP(Amersham Biosciences, Piscataway, NJ, USA), 3 mM Cyanine 3-dCTP (NEN Life Science Products, Boston, MA, USA) (control sample) or 3 mM Cyanine 5-dCTP(NEN Life Science Products, Boston, MA, USA)

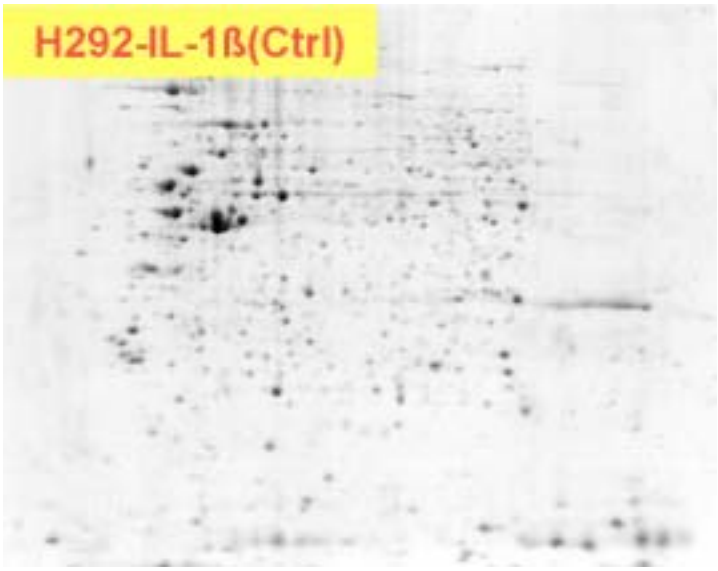
(treated sample). The reaction mixture was mixed by gently flicking the tube and 400 units of SuperScript II™ RNase H-Reverse Transcriptase (Invitrogen life technologies, Carlsbad, CA, USA) was added to the reaction mixture and the reverse transcription allowed to proceed for 2 hrs at 42°C. The reaction was terminated by 5 μℓ stop solution (0.5M NaOH, 50 mM EDTA) and heat inactivation at 65°C for 10 min. Labeled cDNA probe were prepared alcohol precipitation.

Hybridization was carried out on the cDNA chip (8.6K genes, GenoCheck, Ansan, Korea) overnight at 62℃ in a custom slide cassette under humidity maintained by small reservoir of distilled water. Arrays were washed for 2 min with wash buffer 1 (2×SSC, 0.1% SDS), for 3 min with wash buffer 2 (1×SSC), for 2min with wash buffer 3 (0.2×SSC) at room temperature. Gene spots showing more than one and half fold difference between the Cy3 and Cy5 signals were considered as differentially expressed genes by IL-1β induction. The comparison of the data analysis obtained from the two experiments indicated that both experiments were highly reproducible.

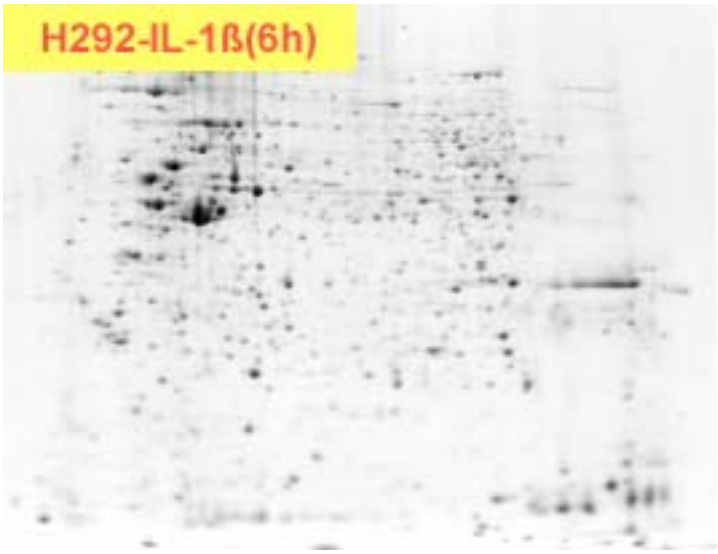
III. RESULTS

1. 2-D PAGE

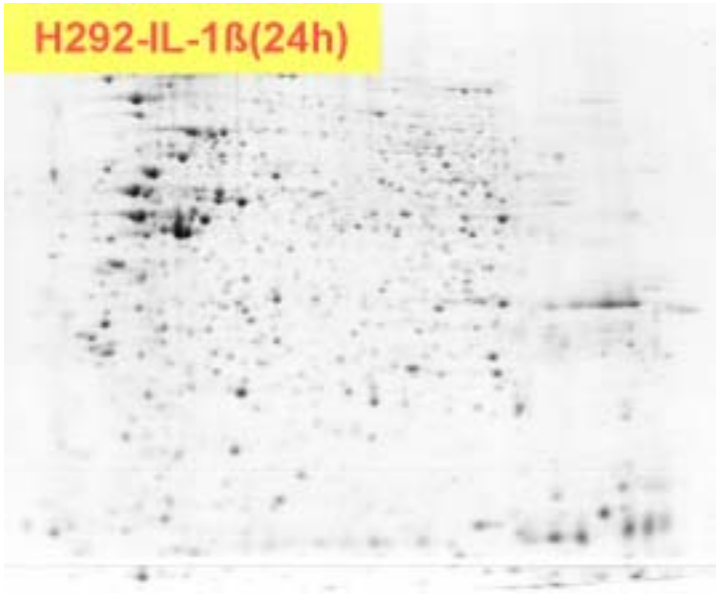
Thirteen hundred well-resolved spots were evident on each pH 3.0 – 10.0 gels (Figure 1). There were 4 up-regulated and 4 down-regulated proteins induced by the 6-hour or 24- hour treatment with IL-1β. Post-translational modifications of proteins, such as changes of molecular weight and pI were observed in 14 proteins. Taken together, the regulated protein induced by IL-1β was 1.7% of the detected protein spots.



A



B



C

Figure 1. Master image of 2-D PAGE gel stained with Coomassie Blue G250. Thirteen hundred discrete spots were visualized with Coomassie blue staining. A: 2-D PAGE gel of control (untreated) NCI-H292 cells. B: 2-D PAGE gel of 6-hour IL-1β-treated NCI-H292 cells. C: 2-D PAGE gel of 24-hour IL-1β-treated NCI-H292 cells.

A. Up-regulated proteins induced by IL-1β in NCI-H292 cells

Up-regulated proteins induced by 6-hour or 24-hour treatment with IL-1β were alpha enolase, heat shock protein 90 beta, tubulin alpha 2, and 2-phosphopyruvate-hydratase alpha-enolase (Table 1) (Figure 2). These proteins were up-regulated in 6-treatment or 24-hour treatment or both.

Table 1. Up-regulated proteins induced by IL-1β

Protien Name	Accession Number	MW (kDa)	pI	Mowse Score	% Coverage
Alpha enolase	P06733	47.169	7.0	1.79E+13	44%
Heat shock protein 90-beta	P08238	83.265	5.0	4.39E+12	28%
Similar to tubulin alpha 2	18204869	37.218	4.9	1.05E+07	34%
2-phosphopyruvate-hydratase enolase; carbonate dehydratase	693933	47.109	7.0	1.19E+04	21%

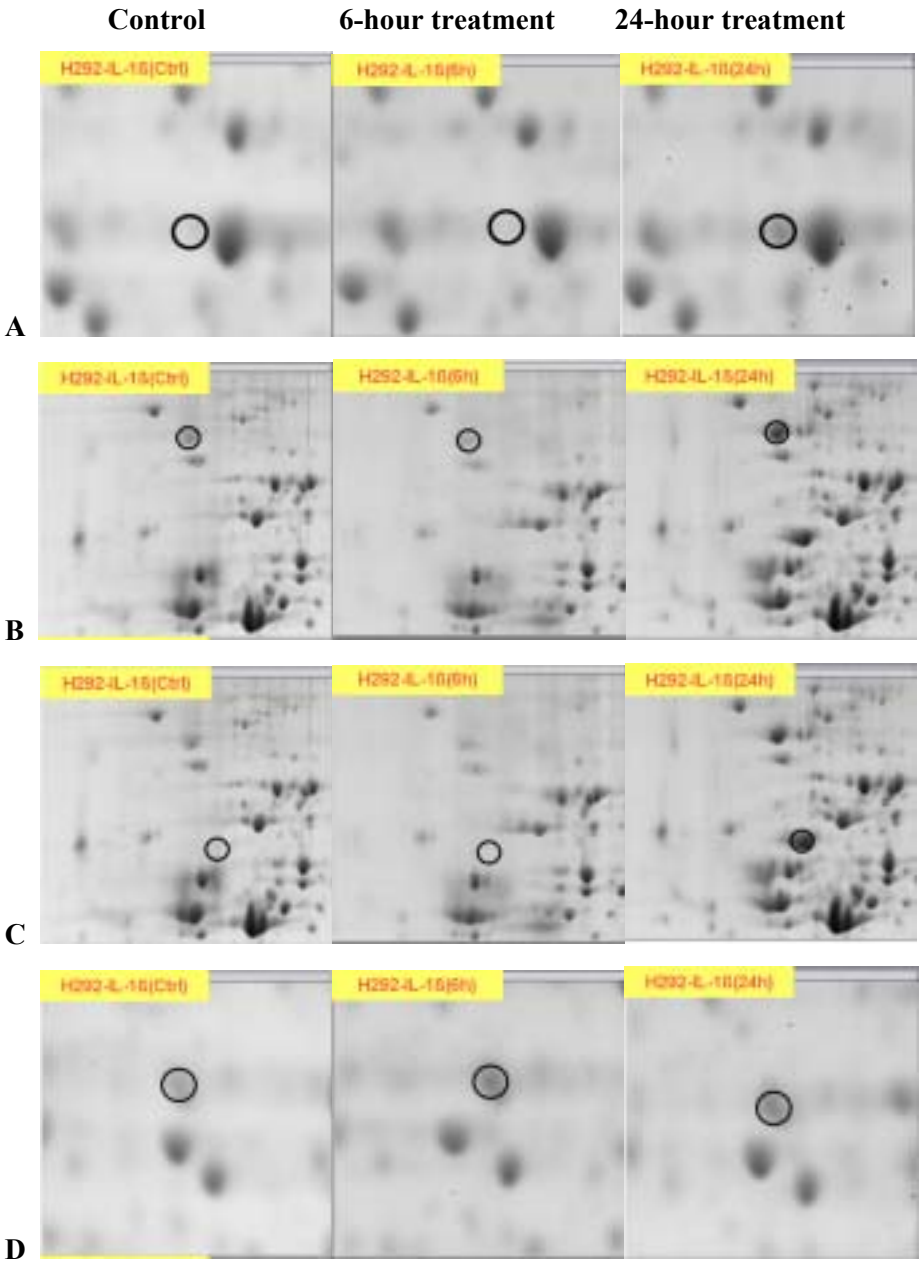


Figure 2. 2-DE images of up-regulated proteins induced by IL-1β in NCI-H292 cells.

A: 2-phosphopyruvate-hydratase alpha-enolase. B: heat shock protein 90-beta. C: similar to tubulin alpha 2. D: alpha enolase.

B. Down-regulated proteins induced by IL-1β in NCI-H292 cells

Down-regulated proteins induced by IL-1β were glutathione S-transferase, thioredoxin peroxidase 1, malate dehydrogenase, and a unnamed product (Table 2) (Figure 3). These proteins were down-regulated in 6-treatment or 24-hour treatment or both.

Table 2. Down-regulated proteins induced by IL-1β

Protien Name	Accession Number	MW (kDa)	pI	Mowse Score	% Coverage
Glutathione S-transferase	2204207	23.382	5.4	1.22E+06	48%
Probable thioredoxin peroxidase 1	2135069	21.891	5.7	1.60E+03	24%
Malate dehydrogenase, cytosolic	7431153	36.415	5.9	1.9E+03	17%
Unnamed protein product	16553410	57.263	6.0	2.36E+03	15%

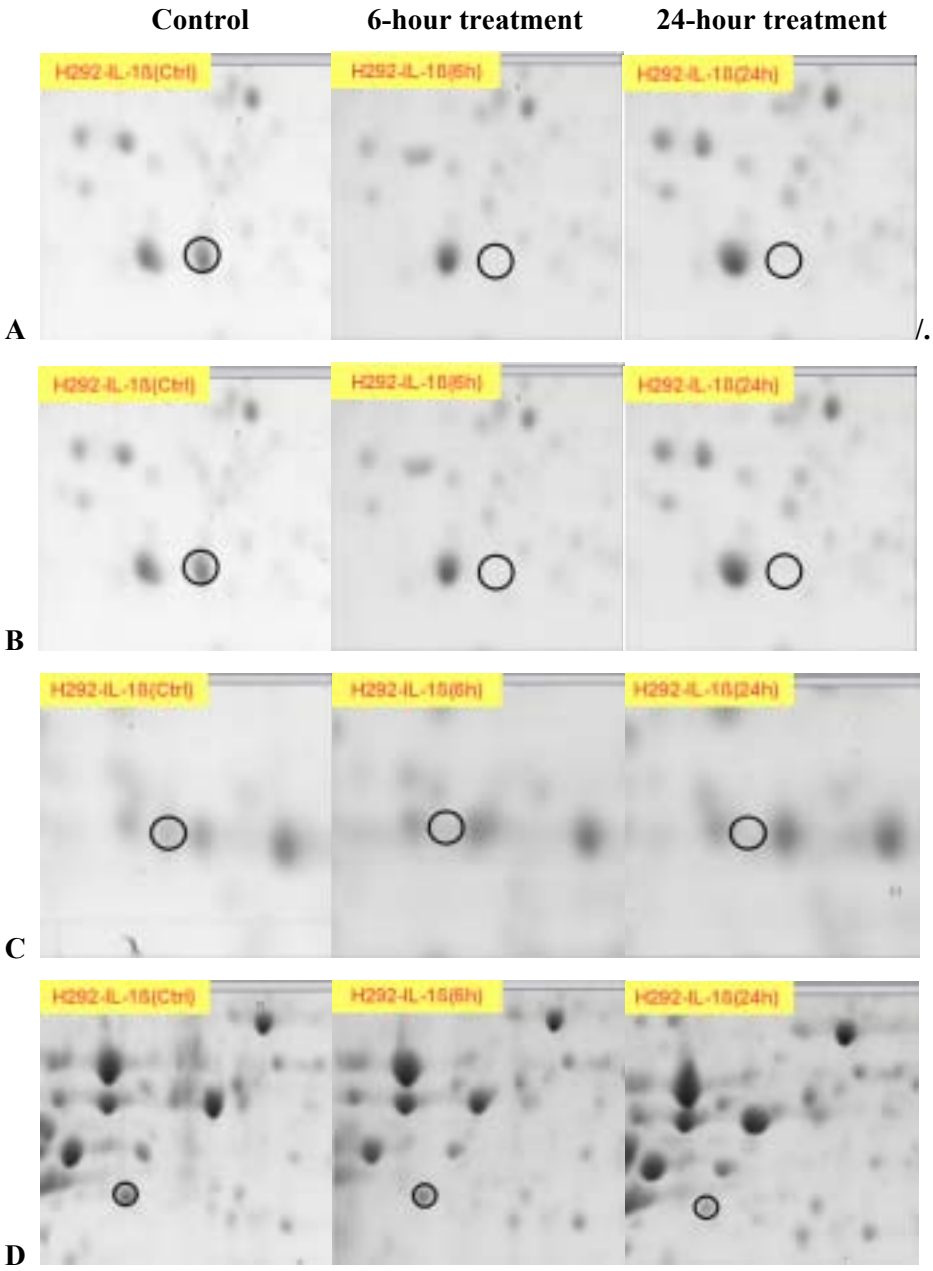


Figure 3. 2-DE images of down-regulated proteins induced by IL-1β in NCI-H292 cells. A: glutathione S-transferase. B: probable thioredoxin peroxidase 1. C: malate dehydrogenase, cytosolic. D: unnamed protein product.

C. Post-translational modification of proteins induced by IL-1β in NCI-H292 cells

Post-translational modification comprised increased molecular weight, decreased molecular weight, and change of pI with the references adjacent several fixed protein spots.

(A) Increased molecular weight

Molecular weights of inorganic pyrophosphatase and erythrocyte cytosolic protein were increased substantially (Table 3) (Figure 4). These spots were located upward compared to control spots.

Table 3. Post-translational modification of proteins induced by IL-1β : Increased molecular weight

Protien Name	Accession Number	MW (kDa)	pI	Mowse Score	% Coverage
Inorganic pyrophophatase (PPase)	Q15181	32.660	5.5	2.26E+04	23%
Erythrocyte cytosolic protein (Reptin52)	5730023	51.157	5.5	7.16E+06	37%

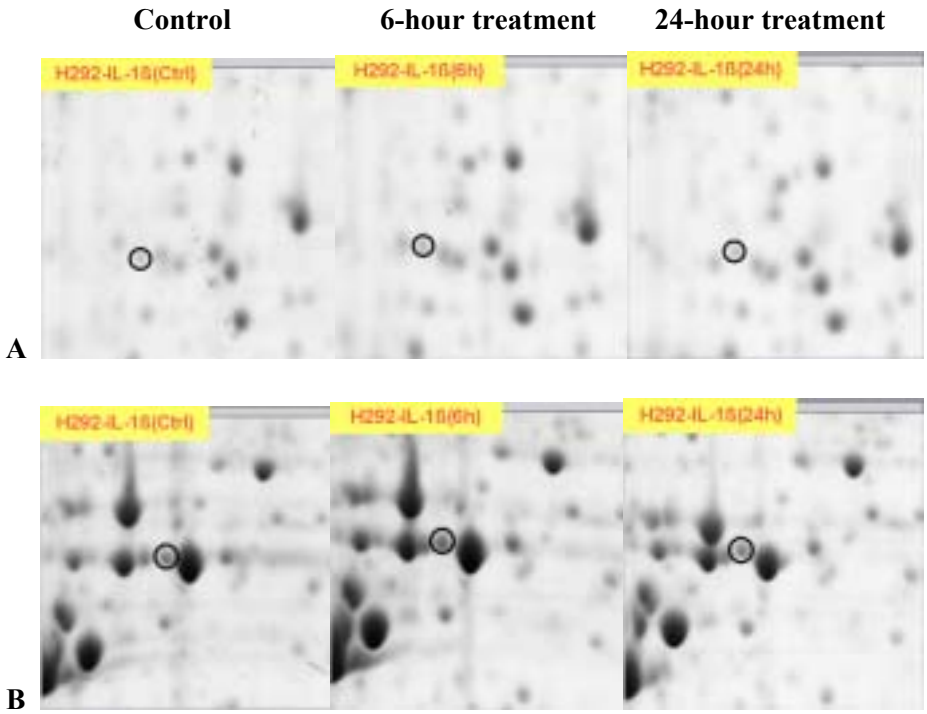


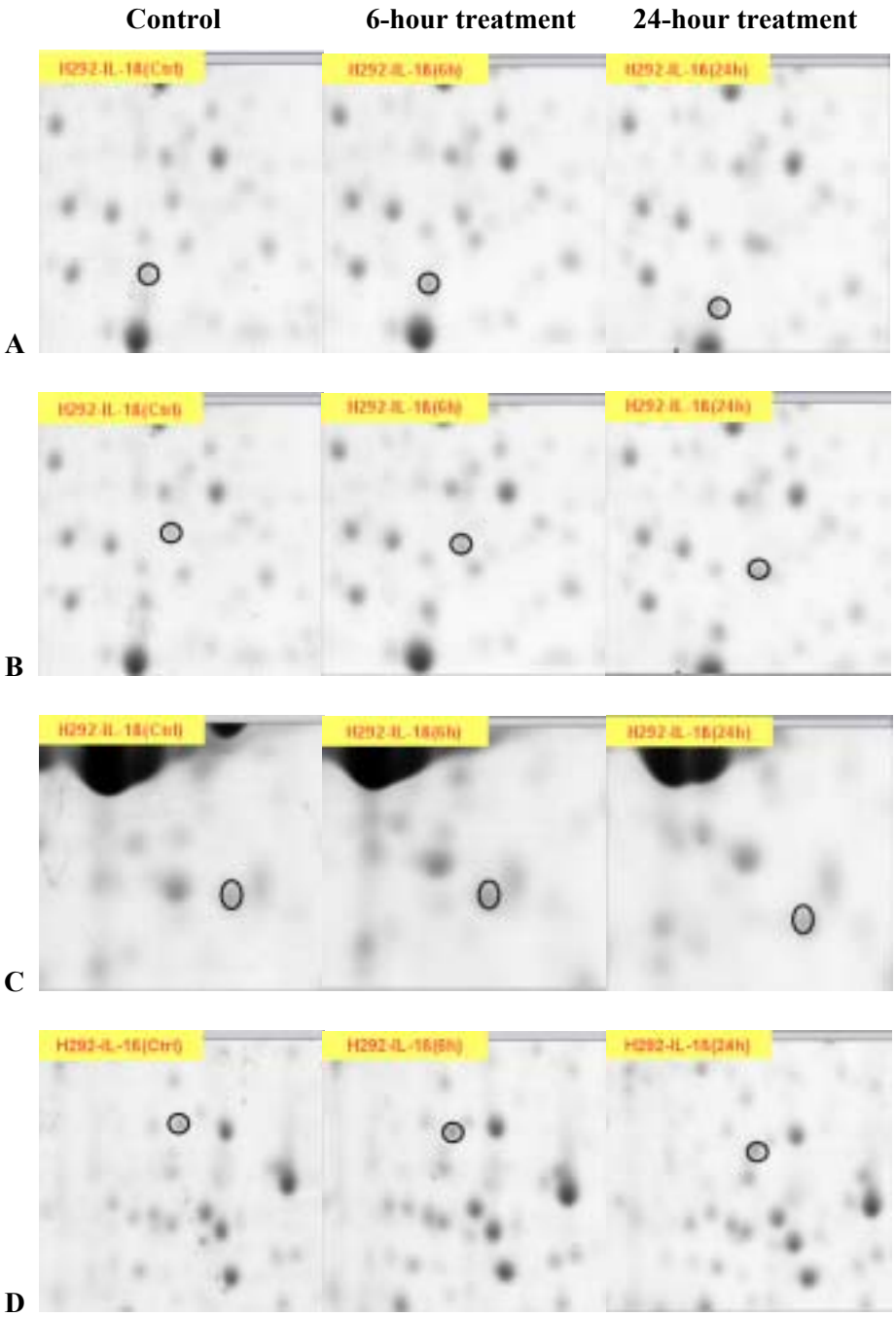
Figure 4. Post-translational modification of proteins induced by IL-1β in NCI-H292 cells : Increased molecular weight. A: inorganic pyrophosphatase. B: erythrocyte cytosolic protein.

(B) Decreased molecular weight

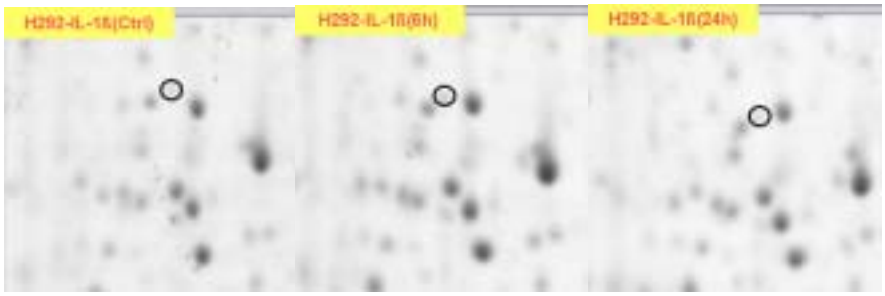
Molecular weight of 9 proteins (heterogenous nuclear ribonucleoproteins C1/C2, ekaryotic translation initiation factor 3 subunit 2, cytokeratin 1, calponin 3, heat shock protein (HSP) 27, platelet-activating factor acetylhydrolase IB beta subunit, GTP-binding nuclear protein RAN, proliferation-associated protein 2G4, and cytoplasmic antiproteinase 3) was decreased (Table 4) (Figure 5). These spots were located downward compared to control spots.

Table 4. Post-translational modification of proteins induced by IL-1β : Decreased molecular weight

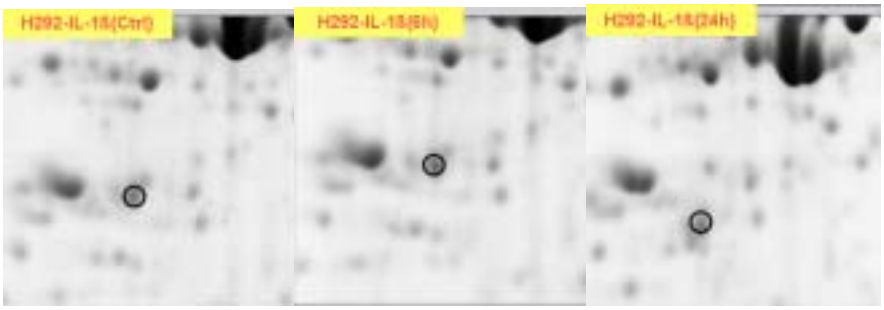
Protien Name	Accession Number	MW (kDa)	pI	Mowse Score	% Coverage
Heterogenous ribonuclear proteins C1/C2	P07910	33.688	5.0	7.42E+04	21%
Eukaryotic translation initiation factor 3 subunit 2 (TGF-beta receptor interacting protein 1)	Q13347	36.502	5.4	2.74E+06	30%
Keratin, type II cytoskeletal 1 (Cytokeratin 1, CK1)	P04264	66.018	8.2	4.26E+06	23%
Calponin, acidic isoform (Calponin 3)	Q15417	36.414	5.7	1.65E+05	19%
Heat shock protein 27 (HSP27)	P04792	22.783	6.0	2.57E+04	33%
Platelet-activating factor acetylhydrolase IB beta subunit	Q29459	25.569	5.6	4.72E+05	39%
GTP-binding nuclear protein RAN (TC4)	P17080	24.423	7.0	7.85E+07	46%
Proliferation-associated protein 2G4	Q9UQ80	43.787	6.1	3.13E+05	20%
Cytoplasmic antiproteinase 3	P50453	42.404	5.6	1.21E+05	25%


Control
6-hour treatment
24-hour treatment
A
B
C
D

E


H292-IL-1ß(Ctrl)
H292-IL-1ß(6h)
H292-IL-1ß(24h)

F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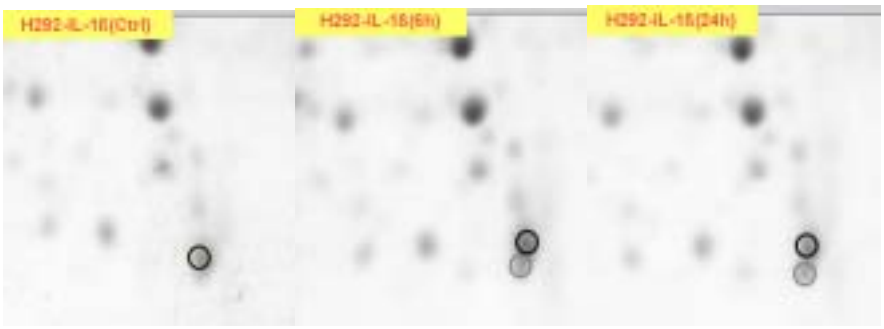
H292-IL-1ß(6h)
H292-IL-1ß(24h)

G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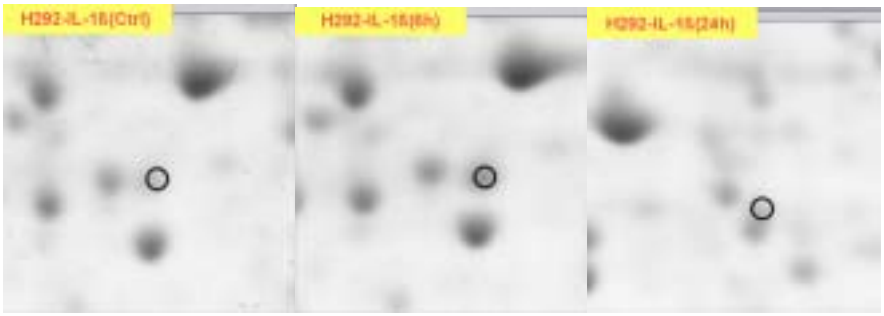
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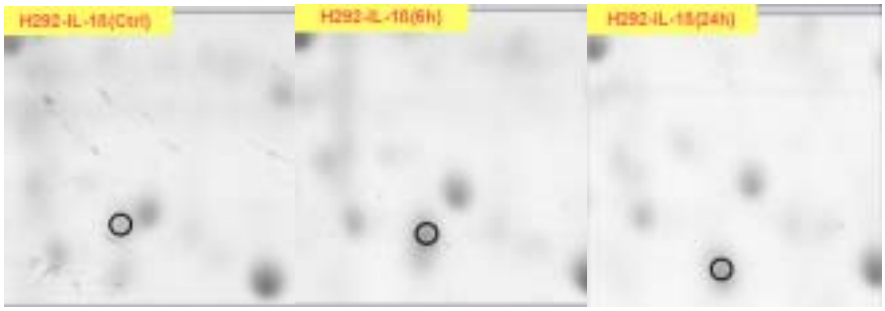
I 

Figure 5. Post-translational modification of proteins induced by IL-1β in NCI-H292 cells : Decreased molecular weight. A: heat shock protein 27. B: platelet-activating factor acetylhydrolase IB beta subunit. C: calponin, acidic isoform. D: eukaryotic translation initiation factor 3 subunit 2. E: keratin, type II cytoskeletal 1. F: heterogeneous nuclear ribonucleoproteins C1/C2. G: GTP-binding nuclear protein RAN. H: proliferation-associated protein 2G4. I: cytoplasmic antiproteinase 3.

(3) Change of pI

pI of 3 proteins (cytokeratin 8, annexin A8, and P43) was changed to acidic (pH 3.0) or to basic (pH 10.0) . Accordingly these spots were move to right or left (Table 5) (Figure 6).

Table. 5. Post-translational modification of proteins induced by IL-1β : Change of pI

Protien Name	Accession Number	MW (kDa)	pI	Mowse Score	% Coverage
Cytokeratin 8	87303	53.563	5.5	9.04E+05	31%
Annexin VIII (Annexin A8)	71772	36.895	5.6	1.88E+05	29%
P43	2119918	49.534	7.7	5.43E+03	17%

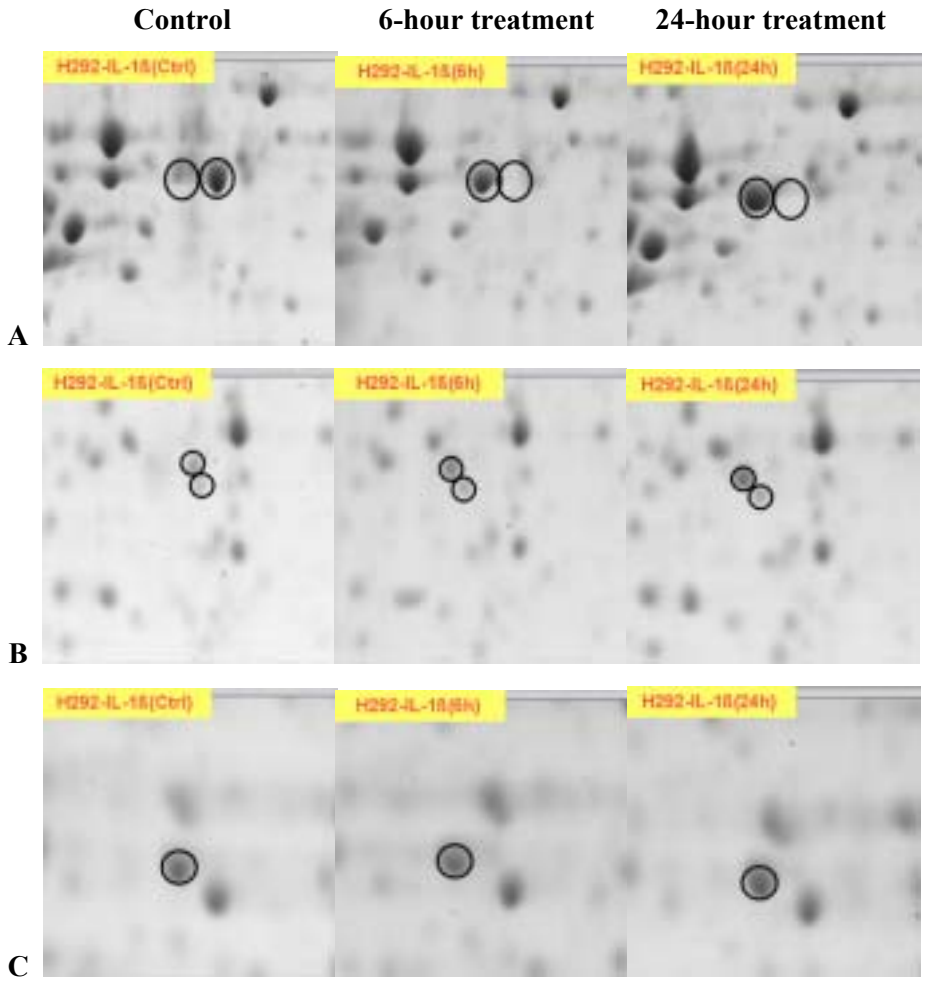
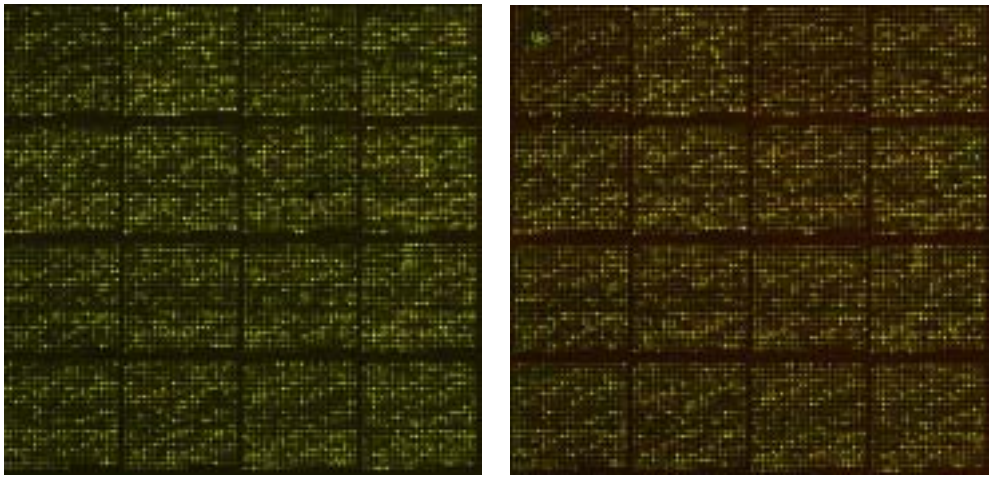


Figure 6. Post-translational modification of proteins induced by IL-1β : Change of pI. A: cytokeratin 8. B: annexin VIII. C: P43.

2. cDNA microarray

Among the total 8672 genes placed on the chips, 6230 genes (71.8%) were detected (Figure 7A) and 413 genes (6.6%) were up-regulated or down-regulated in the IL-1β 6-hour-treated NCI-H292 cells. In the IL-1β 24-hour-treated NCI-H292 cells, 5729 genes (66.1%) were detected (Figure 7B) and 115 genes (2.0%) were up-regulated or down-regulated.



A B

Figure 7. c-DNA microarray of IL-1β 6-hour-treated (A) and 24-hour-treated (B) NCI-H292 cells. The microarray contains 8672 human genes. Messenger RNA from both control and IL-1β-treated NCI-H292 cells were labeled with Cy3 or Cy5-dUTP, respectively.

The comparative intensity of gene expression for control and IL-1β-treated NCI-H292 cells was shown with the fluorescence intensity of Cy3 on X-axis and Cy5 on Y-axis, respectively (Figure 8). Red spots indicate up-regulated genes, green spots indicate down-regulated genes, yellow spots indicate no changes in expression, and blanks indicate no expression.

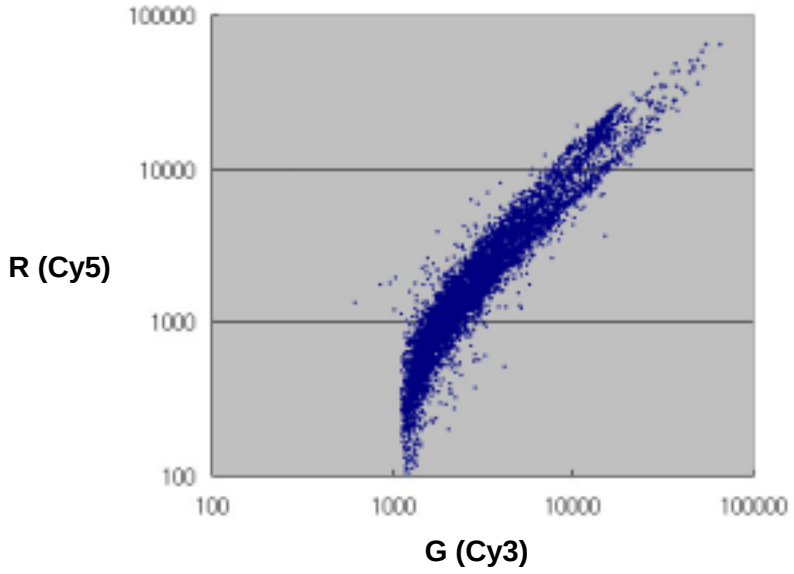


Figure 8. Scatter plot of c-DNA microarray of 24-hour IL-1β treated NCI-H292 cells. The number of up-regulated genes (Cy5/Cy3 > 1.5) was 80 and that of down-regulated genes (Cy5/Cy3 < 0.67) was 35.

In the 6-hour IL-1β-treated NCI-H292 cells, 152 genes were up-regulated (Table 6) in the 6230 detected genes and down-regulated genes were 261 (Table 7). In the 24-hour IL-1β-treated NCI-H292 cells, 80 genes were up-regulated (Table 8) and 35 genes were down-regulated (Table 9). Each gene was categorized by main pathway (metabolic or regulatory pathway) and sub-pathway (Table 10-13). Differentially expressed genes were mostly in the metabolic pathway, such as carbohydrate and amino acid metabolism.

Table 6. Up-regulated gene profile of 6-hour IL-1β treated NCI-H292 cells

Name	ID	Intensity(R/G)
serine (or cysteine) proteinase inhibitor, clade E	N59721	6.61
chemokine (C-X-C motif) ligand 1	W42723	3.93
lactate dehydrogenase B	BQ431769	3.58
Homo sapiens mRNA; cDNA DKFZp547C136 (from clone DKFZp547C136)	BQ130042	3.39
pseudo-chlordecone reductase	R93124	3.07
aldo-keto reductase family 1, member C2	AI924357	2.92
GPI-anchored metastasis-associated protein homolog	AA479609	2.83
ribosomal protein, large P2	N47717	2.73
unnamed protein product [Homo sapiens], mRNA sequence	AA465386	2.53
unknown	No data	2.51
B-factor, properdin	AA401441	2.46
v-myc myelocytomatosis viral oncogene homolog (avian)	V00568	2.43
tissue factor pathway inhibitor 2	AA399473	2.42
gap junction protein, beta 1, 32kDa (connexin 32)	N62394	2.38
glutathione S-transferase A3	N30096	2.30
ESTs, highly similar to I56326 fatty acid binding protein homolog	AI359037	2.29
unknown	No data	2.29
chromosome 17, clone hRPC.1073_F_15	No data	2.27
ubiquitin D	N33920	2.26
ubiquitin carboxyl-terminal esterase L3 (ubiquitin thiolesterase)	M30496	2.09
inhibitor of apoptosis protein-1(MIHC) mRNA	BG875391	2.08
annexin A3	AI949576	2.07
unknown	W86100	2.07
cutaneous T-cell lymphoma-associated tumor antigen se20-4	AI969825	2.06
S-adenosylmethionine decarboxylase 1	R82299	2.01
solute carrier family 1	AA453742	2.00

Table 7. Down-regulated gene profile of 6-hour IL-1β treated NCI-H292 cells

Name	ID	Intensity(R/G)
v-erb-b2 erythroblastic leukemia viral oncogene homolog 2	AA446928	0.23
ESTs, highly similar to CA17_HUMAN cdollagen alpha 1(VII) chain	AA598507	0.32
iroquois homeobox protein 5	R46202	0.34
unknown	No data	0.35
disabled homolog 2, mitogen-responsive phosphoprotein	AA448656	0.37
Homo sapiens cDNA FLJ32541 fis, clone SMINT2000530, mRNA sequence	AI004484	0.37
N-acylaminoacyl-peptide hydrolase	D38441	0.37
mucin 4, tracheobronchial	AI884498	0.38
nucleosome assembly protein 1-like 4	H92201	0.40
SH2-B homolog	N94482	0.40
peroxisomal biogenesis factor 16	AI654715	0.42
calreticulin	H99170	0.43
hypoxia up-regulated 1	AA099134	0.43
melanoma antigen, family D	AW073182	0.44
keratin 8	AA598517	0.44
butyrophilin, subfamily 3, member A1	N66053	0.44
parathyroid hormone receptor 1	AA872602	0.45
ADP-ribosylation factor 4-like	H15085	0.45
glucosidase, beta (bile acid) 2	AA461304	0.45
unknown	No data	0.46
hypothetical protein MGC1203	AA975458	0.46
dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive)	AI310142	0.46
splicing factor, arginine/serine-rich 5	T90980	0.49
aldehyde dehydrogenase 3 family, member B2	AA443630	0.49
nucleolar autoantigen (55kD)	U47621	0.49
solute carrier family 25 (mitochondrial carrier)	J03592	0.50
Orf1, mRNA sequence	H91303	0.50
chloride channel 6	H08188	0.50

Table 8. Up-regulated gene profile of 24-hour IL-1β treated NCI-H292 cells

Name	ID	Intensity(R/G)
solute carrier family 28 (sodium-coupled nucleoside transporter)	AI344386	18.49
B-factor, properdin	AA401441	6.87
ubiquitin D	N33920	6.83
B-factor, properdin	AA401441	5.15
chemokine (C-X-C motif) ligand 1	W42723	4.71
lactate dehydrogenase B	BQ431769	3.75
annexin A13	AA884167	3.65
gap junction protein, beta 1, 32kDa	N62394	3.52
glutathione S-transferase A3	N30096	2.49
lipocalin 2 (oncogene 24p3)	AA400973	2.37
tumor necrosis factor, alpha-induced protein 3	AA476272	2.24
serine (or cysteine) proteinase inhibitor, clade B (ovalbumin)	AA398883	2.21
forkhead box O3A	AA465236	2.13
CDC28 protein kinase regulatory subunit 2	AA397813	2.05
aldo-keto reductase family 1, member C2	AI924357	2.03
nuclear factor of kappa light polypeptide gene enhancer in B-cell	W56300	2.02
BRCA2 and CDKN1A interacting protein	T86027	2.02

Table 9. Down-regulated gene profile of 24-hour IL-1β treated NCI-H292 cells

Name	ID	Intensity(R/G)
hypothetical protein MGC1203	AA975458	0.50
unknown	No data	0.51
ESTs, highly similar to CA17_HUMAN Collagen alpha 1(VII) chain	AA598507	0.52
phospholipase A2 receptor 1, 180kDa	AA086038	0.54
microtubule-associated protein 4	AA130870	0.55
protein geranylgeranyltransferase type I, beta subunit	AA961272	0.56
ADP-ribosylation factor 4-like	H15085	0.58
collagen, type X, alpha 1 (Schmid metaphyseal chondrodysplasia)	AI828306	0.58
parathyroid hormone receptor 1	AA872602	0.58
p53-induced protein	H11660	0.59
putative c-Myc-responsive	AA132086	0.59
Homo sapiens cDNA FLJ32541 fis, clone SMINT2000530, mRNA sequence	AI004484	0.60
chloride channel 6	H08188	0.61
major vault protein	AA158991	0.61
solute carrier family 25 (mitochondrial carrier)	J03592	0.62
hypothetical protein FLJ14675	AA485743	0.63
transient receptor potential cation channel, subfamily V	W88571	0.63
nuclear factor of kappa light polypeptide gene enhancer in B-cell	AA437370	0.63
dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive)	AI310142	0.63
phosphomevalonate kinase	H09819	0.64
keratin, hair, acidic, 2	AI190798	0.64
folate receptor 2 (fetal)	AA453816	0.64
similar to S. cerevisiae Sec6p and R. norvegicus rsec6	AA917609	0.64
SH2-B homolog	N94482	0.64
pregnancy specific beta-1-glycoprotein 4	N30553	0.64
hypothetical protein FLJ22301	AA458867	0.65
chloride intracellular channel 1	AA485913	0.65
unknown	No data	0.65
ESTs, Highly similar to A55933 paxillin - human [H.sapiens]	AA430574	0.65
keratin 19	AA464250	0.65
ribosomal protein L13a	X56932	0.66
Notch homolog 3 (Drosophila)	AA481418	0.66
MLL septin-like fusion	AW004927	0.66
B-cell CLL/lymphoma 9	AA866054	0.66
keratin 8	AA598517	0.67

Table 10. Clustering of up-regulated gene profile of 6-hour IL-1β treated NCI-H292 cells by pathway

Main path	Sub path	Name	Intensity (R/G)
Amino Acid Metabolism	Cysteine metabolism	lactate dehydrogenase B	3.5765
	Arginine and proline metabolism	S-adenosylmethionine decarboxylase 1	2.0081
	Cysteine metabolism	sulfotransferase family 4A, member 1	1.9053
	Lysine degradation	trimethyllysine hydroxylase, epsilon	1.6000
	Arginine and proline metabolism	arginyl-tRNA synthetase	1.5682
	Glycine, serine and threonine metabolism	glycyl-tRNA synthetase	1.5574
	Tryptophan metabolism	tryptophanyl-tRNA synthetase	1.5031
Biodegradation of Xenobiotics	Ethylbenzene degradation	solute carrier family 27 (fatty acid transporter)	1.8671
	Benzoate degradation via CoA ligation	serine threonine kinase 39	1.7244
Carbohydrate Metabolism	Glycolysis / Gluconeogenesis	lactate dehydrogenase B	3.5765
	Pyruvate metabolism	lactate dehydrogenase B	3.5765
	Propanoate metabolism	lactate dehydrogenase B	3.5765
	Propanoate metabolism	solute carrier family 27 (fatty acid transporter), member 2	1.8671
	Glyoxylate and dicarboxylate metabolism	methylene tetrahydrofolate dehydrogenase (NAD+ dependent)	1.7689
	Glyoxylate and dicarboxylate metabolism	methylene tetrahydrofolate dehydrogenase (NAD+ dependent)	1.4987
Cell Growth and Death	Cell cycle	CDC6 cell division cycle 6 homolog	1.5594
	Cell cycle	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation	1.5430
	Cell cycle	MAD2 mitotic arrest deficient-like 1 (yeast)	1.5218
Energy Metabolism	Oxidative phosphorylation	NADH dehydrogenase (ubiquinone) Fe-S protein 3, 30kDa	1.7107
Metabolism of Cofactors and Vitamins	Nicotinate and nicotinamide metabolism	nucleoside phosphorylase	1.9912
	One carbon pool by folate	methylene tetrahydrofolate dehydrogenase (NAD+ dependent)	1.7689
	Nicotinate and nicotinamide metabolism	serine threonine kinase 39 (STE20/SPS1 homolog, yeast)	1.7244
	Ubiquinone biosynthesis	NADH dehydrogenase (ubiquinone) Fe-S protein 3	1.7107
	One carbon pool by folate	methylene tetrahydrofolate dehydrogenase	1.4987
Metabolism of Complex Carbohydrates	Starch and sucrose metabolism	serine threonine kinase 39 (STE20/SPS1 homolog, yeast)	1.7244
Metabolism of Complex Lipids	Inositol phosphate metabolism	serine threonine kinase 39 (STE20/SPS1 homolog, yeast)	1.7244
	Sphingoglycolipid metabolism	serine threonine kinase 39 (STE20/SPS1 homolog, yeast)	1.7244
Metabolism of Other Amino Acids	Glutathione metabolism	glutathione S-transferase A3	2.3008
	Glutathione metabolism	microsomal glutathione S-transferase 1	1.5283
Neurodegenerative Disorders	Prion disease	prion protein (p27-30) (Creutzfeld-Jakob disease)	1.7978
	Prion disease	prion protein (p27-30) (Creutzfeld-Jakob disease)	1.5895
	Prion disease	nuclear factor (erythroid-derived 2)-like 2	1.5450
	Alzheimer's disease	guanine nucleotide binding protein (G protein)	1.5052

Nucleotide Metabolism	Purine metabolism	nucleoside phosphorylase	1.9912
	Pyrimidine metabolism	nucleoside phosphorylase	1.9912
	Purine metabolism	phosphodiesterase 4D, cAMP-specific	1.6092
	Purine metabolism	polymerase (RNA) II (DNA directed) polypeptide K	1.5934
	Pyrimidine metabolism	polymerase (RNA) II (DNA directed) polypeptide K	1.5934
	Purine metabolism	hypoxanthine phosphoribosyltransferase 1	1.5544
	Purine metabolism	adenylate kinase 2	1.5269
	Purine metabolism	adenylate kinase 3	1.4952
Sorting and Degradation	Proteasome	proteasome (prosome, macropain) subunit, alpha type, 3	1.4972
Transcription	RNA polymerase	polymerase (RNA) II (DNA directed) polypeptide K	1.5934
Translation	Aminoacyl-tRNA biosynthesis	arginyl-tRNA synthetase	1.5682
	Aminoacyl-tRNA biosynthesis	glycyl-tRNA synthetase	1.5574
	Aminoacyl-tRNA biosynthesis	tryptophanyl-tRNA synthetase	1.5031

Table 11. Clustering of down-regulated gene profile of 6-hour IL-1β treated NCI-H292 cells by pathway

Main path	Sub path	Name	Intensity (R/G)
Amino Acid Metabolism	Histidine metabolism	histamine N-methyltransferase	0.6591
	Histidine metabolism	aldehyde dehydrogenase 3 family, member B2	0.4920
	Tyrosine metabolism	aldehyde dehydrogenase 3 family, member B2	0.4920
	Phenylalanine metabolism	aldehyde dehydrogenase 3 family, member B2	0.4920
	Glutamate metabolism	glutamate-ammonia ligase(glutamine synthase)	0.5854
	Valine, leucine and isoleucine degradation	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase	0.6037
	Valine, leucine and isoleucine degradation	aldehyde dehydrogenase 1 family, member A1	0.5944
	Lysine degradation	aldehyde dehydrogenase 1 family, member A1	0.5944
	Arginine and proline metabolism	aldehyde dehydrogenase 1 family, member A1	0.5944
	Histidine metabolism	aldehyde dehydrogenase 1 family, member A1	0.5944
	Tryptophan metabolism	aldehyde dehydrogenase 1 family, member A1	0.5944
Biodegradation of Xenobiotics	Benzoate degradation via CoA ligation	protein kinase C, delta	0.5853
	Benzoate degradation via CoA ligation	mitogen-activated protein kinase 10	0.6377
	Benzoate degradation via CoA ligation	ribosomal protein S6 kinase, 70kDa, polypeptide 2	0.6699
	Benzoate degradation via CoA ligation	cyclin-dependent kinase (CDC2-like) 10	0.6669
	1,2-Dichloroethane degradation	aldehyde dehydrogenase 1 family, member A1	0.5944
Biosynthesis of Secondary Metabolites	Streptomycin biosynthesis	myo-inositol 1-phosphate synthase A1	0.6018
Carbohydrate Metabolism	Citrate cycle (TCA cycle)	citrate synthase	0.5569
	Glyoxylate and dicarboxylate metabolism	citrate synthase	0.5569
	Glycolysis / Gluconeogenesis	aldehyde dehydrogenase 3 family, member B2	0.4920
	Glycolysis / Gluconeogenesis	glyceraldehyde-3-phosphate dehydrogenase	0.6262
	Butanoate metabolism	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase	0.6037
	Glycolysis / Gluconeogenesis	aldehyde dehydrogenase 1 family, member A1	0.5944
	Ascorbate and aldarate metabolism	aldehyde dehydrogenase 1 family, member A1	0.5944
	Pyruvate metabolism	aldehyde dehydrogenase 1 family, member A1	0.5944
	Propanoate metabolism	aldehyde dehydrogenase 1 family, member A1	0.5944
	Butanoate metabolism	aldehyde dehydrogenase 1 family, member A1	0.5944
	Citrate cycle (TCA cycle)	citrate synthase	0.6143
	Glyoxylate and dicarboxylate metabolism	citrate synthase	0.6143

Cell Communication	Integrin-mediated cell adhesion	paxillin	0.5963
	Integrin-mediated cell adhesion	selenoprotein P, plasma, 1	0.6566
	Integrin-mediated cell adhesion	SHC (Src homology 2 domain containing) transforming protein 1	0.5941
Cell Growth and Death	Apoptosis	nuclear factor of kappa light polypeptide gene enhancer in B-cell	0.5103
Energy Metabolism	Oxidative phosphorylation	NADH dehydrogenase (ubiquinone) flavoprotein 1	0.6317
	Nitrogen metabolism	glutamate-ammonia ligase (glutamine synthase)	0.5854
	Oxidative phosphorylation	ATP synthase, H+ transporting, mitochondrial F0 complex	0.6262
	ATP synthesis	ATP synthase, H+ transporting, mitochondrial F0 complex	0.6262
Lipid Metabolism	Sterol biosynthesis	phosphomevalonate kinase	0.5056
	Sterol biosynthesis	EST	0.6320
	Synthesis and degradation of ketone bodies	3-hydroxymethyl-3-methylglutaryl-Coenzyme A lyase	0.6037
	Fatty acid metabolism	aldehyde dehydrogenase 1 family, member A1	0.5944
	Bile acid biosynthesis	aldehyde dehydrogenase 1 family, member A1	0.5944
Metabolism of Cofactors and Vitamins	Nicotinate and nicotinamide metabolism	protein kinase C, delta	0.5853
	Nicotinate and nicotinamide metabolism	mitogen-activated protein kinase 10	0.6377
	Nicotinate and nicotinamide metabolism	ribosomal protein S6 kinase, 70kDa, polypeptide 2	0.6699
	Nicotinate and nicotinamide metabolism	cyclin-dependent kinase (CDC2-like) 10	0.6669
	Ubiquinone biosynthesis	NADH dehydrogenase (ubiquinone) flavoprotein 1	0.6317
Metabolism of Complex Carbohydrates	Chondroitin / Heparan sulfate biosynthesis	uronyl-2-sulfotransferase	0.6682
	Starch and sucrose metabolism	protein kinase C, delta	0.5853
	Starch and sucrose metabolism	mitogen-activated protein kinase 10	0.6377
	Starch and sucrose metabolism	ribosomal protein S6 kinase, 70kDa, polypeptide 2	0.6699
	Starch and sucrose metabolism	cyclin-dependent kinase (CDC2-like) 10	0.6669
	O-Glycans biosynthesis	sialyltransferase 4C (beta-galactoside alpha-2,3-sialytransferase	0.5777
	Peptidoglycan biosynthesis	glutamate-ammonia ligase (glutamine synthase)	0.5854
Metabolism of Complex Lipids	Inositol phosphate metabolism	protein kinase C, delta	0.5853
	Sphingoglycolipid metabolism	protein kinase C, delta	0.5853
	Inositol phosphate metabolism	mitogen-activated protein kinase 10	0.6377
	Sphingoglycolipid metabolism	mitogen-activated protein kinase 10	0.6377
	Inositol phosphate metabolism	ribosomal protein S6 kinase, 70kDa, polypeptide 2	0.6699

Metabolism of Complex Lipids	Sphingoglycolipid metabolism	ribosomal protein S6 kinase, 70kDa, polypeptide 2	0.6699
	Inositol phosphate metabolism	myo-inositol 1-phosphate synthase A1	0.6018
	Inositol phosphate metabolism	cyclin-dependent kinase (CDC2-like) 10	0.6669
	Sphingoglycolipid metabolism	cyclin-dependent kinase (CDC2-like) 10	0.6669
	Inositol phosphate metabolism	phosphatidylinositol-4-phosphate 5-kinase, type I, alpha	0.6286
	Globoside metabolism	sialyltransferase 4C (beta-galactoside alpha-2,3-sialytransferase	0.5777
	Sphingoglycolipid metabolism	arylsulfatase A	0.6210
	Glycerolipid metabolism	aldehyde dehydrogenase 1 family, member A1	0.5944
Metabolism of Other Amino Acids	D-Arginine and D-ornithine metabolism	glyceraldehyde-3-phosphate dehydrogenase	0.6262
	beta-Alanine metabolism	aldehyde dehydrogenase 1 family, member A1	0.5944
Neurodegenerative Disorders	Alzheimer's disease	glyceraldehyde-3-phosphate dehydrogenase	0.6262
	Huntington's disease	glyceraldehyde-3-phosphate dehydrogenase	0.6262
	Dentatorubropallidoluysian atrophy (DRPLA)	glyceraldehyde-3-phosphate dehydrogenase	0.6262
	Alzheimer's disease	guanine nucleotide binding protein (G protein), beta 5	0.6689
	Alzheimer's disease	amyloid beta (A4) precursor protein	0.6628
Nucleotide Metabolism	Purine metabolism	guanylate cyclase 1, soluble, beta 3	0.6338
	Purine metabolism	IMP (inosine monophosphate) dehydrogenase 2	0.5726
Signal Transduction	Phosphatidylinositol signaling system	protein tyrosine phosphatase, non-receptor type 21	0.6596
	Phosphatidylinositol signaling system	protein kinase C, zeta	0.5065
	Phosphatidylinositol signaling system	protein kinase C, delta	0.5853
	MAPK signaling pathway	mitogen-activated protein kinase 10	0.6377
	Phosphatidylinositol signaling system	phosphatidylinositol-4-phosphate 5-kinase, type I, alpha	0.6286
	MAPK signaling pathway	SHC transforming protein 1	0.5941
	MAPK signaling pathway	GRB2-related adaptor protein 2	0.6582
Sorting and Degradation	Ubiquitin mediated proteolysis	F-box and WD-40 domain protein 1B	0.6607
Transcription	Transcription factors	general transcription factor IIF, polypeptide 1	0.5907
Translation	Ribosome	ribosomal protein L13a	0.6666
	Ribosome	ribosomal protein L13a	0.6094

Table 12. Clustering of up-regulated gene profile of 24-hour IL-1β treated NCI-H292 cells by pathway

Main path	Sub path	Name	Intensity (R/G)
Amino Acid Metabolism	Cysteine metabolism	sulfotransferase family 4A, member 1	1.9476
	Cysteine metabolism	lactate dehydrogenase B	3.7542
	Methionine metabolism	DNA (cytosine-5-)-methyltransferase 1	1.5057
Behavior	Circadian rhythm	aryl hydrocarbon receptor nuclear translocator	1.5793
Biodegradation of Xenobiotics	Benzoate degradation via CoA ligation	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.6441
	Benzoate degradation via CoA ligation	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.6595
	Benzoate degradation via CoA ligation	polo-like kinase (Drosophila)	1.7204
	Benzoate degradation via CoA ligation	TTK protein kinase	1.7002
	Benzoate degradation via CoA ligation	polo-like kinase (Drosophila)	1.7360
	Benzoate degradation via CoA ligation	TTK protein kinase	1.9303
Biosynthesis of Secondary Metabolites	Streptomycin biosynthesis	inositol(myo)-1(or 4)-monophosphatase 1	1.6230
Carbohydrate Metabolism	Glycolysis / Gluconeogenesis	lactate dehydrogenase B	3.7542
	Pyruvate metabolism	lactate dehydrogenase B	3.7542
	Propanoate metabolism	lactate dehydrogenase B	3.7542
Cell Growth and Death	Cell cycle	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.6441
	Cell cycle	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.6595
	Cell cycle	polo-like kinase (Drosophila)	1.7204
	Cell cycle	MAD2 mitotic arrest deficient-like 1 (yeast)	1.7453
	Cell cycle	cyclin A2	1.7065
	Apoptosis	nuclear factor of kappa light polypeptide gene enhancer in B-cell	2.0242
	Cell cycle	pituitary tumor-transforming 1	1.9182
	Cell cycle	polo-like kinase (Drosophila)	1.7360
	Apoptosis	tumor necrosis factor receptor superfamily, member 6	1.5988
Lipid Metabolism	C21-Steroid hormone metabolism	hydroxy-delta-5-steroid dehydrogenase	1.5603
	Androgen and estrogen metabolism	hydroxy-delta-5-steroid dehydrogenase	1.5603
	C21-Steroid hormone metabolism	hydroxysteroid (11-beta) dehydrogenase 1	1.6990
	Androgen and estrogen metabolism	hydroxysteroid (11-beta) dehydrogenase 1	1.6990
Metabolism of Cofactors and Vitamins	Nicotinate and nicotinamide metabolism	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.6441
	Nicotinate and nicotinamide metabolism	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.6595
	Nicotinate and nicotinamide metabolism	polo-like kinase (Drosophila)	1.7204
	Nicotinate and nicotinamide metabolism	nicotinamide N-methyltransferase	1.8644

Metabolism of Cofactors and Vitamins	Nicotinate and nicotinamide metabolism	TTK protein kinase	1.7002
	Nicotinate and nicotinamide metabolism	polo-like kinase (Drosophila)	1.7360
	Nicotinate and nicotinamide metabolism	TTK protein kinase	1.9303
Metabolism of Complex Carbohydrates	Starch and sucrose metabolism	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.6441
Metabolism of Complex Lipids	Starch and sucrose metabolism	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.6595
	Starch and sucrose metabolism	polo-like kinase (Drosophila)	1.7204
	Starch and sucrose metabolism	TTK protein kinase	1.7002
	Starch and sucrose metabolism	polo-like kinase (Drosophila)	1.7360
	Starch and sucrose metabolism	TTK protein kinase	1.9303
	Inositol phosphate metabolism	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.6441
Metabolism of Other Amino Acids	Sphingoglycolipid metabolism	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.6441
	Inositol phosphate metabolism	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.6595
	Sphingoglycolipid metabolism	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.6595
	Inositol phosphate metabolism	polo-like kinase (Drosophila)	1.7204
	Sphingoglycolipid metabolism	polo-like kinase (Drosophila)	1.7204
	Glycerolipid metabolism	platelet-activating factor acetylhydrolase 2, 40kDa	1.5048
	Inositol phosphate metabolism	inositol(myo)-1(or 4)-monophosphatase 1	1.6230
	Inositol phosphate metabolism	TTK protein kinase	1.7002
	Sphingoglycolipid metabolism	TTK protein kinase	1.7002
	Inositol phosphate metabolism	polo-like kinase (Drosophila)	1.7360
	Sphingoglycolipid metabolism	polo-like kinase (Drosophila)	1.7360
	Inositol phosphate metabolism	TTK protein kinase	1.9303
	Sphingoglycolipid metabolism	TTK protein kinase	1.9303
	Glutathione metabolism	glutathione S-transferase A3	2.4896
Nucleotide Metabolism	Purine metabolism	polymerase (RNA) II (DNA directed) polypeptide K	1.5481
Signal Transduction	Pyrimidine metabolism	polymerase (RNA) II (DNA directed) polypeptide K	1.5481
	Phosphatidylinositol signaling system	inositol (myo)-1(or 4)-monophosphatase 1	1.6230
Transcription	Phosphatidylinositol signaling system	dual specificity phosphatase 3	1.7566
	RNA polymerase	polymerase (RNA) II (DNA directed) polypeptide K	1.5481
Translation	Ribosome	ribosomal protein S21	1.5236

Table 13. Clustering of down-regulated gene profile of 24-hour IL-1β treated NCI-H292 cells by pathway

Main path	Sub path	Name	Intensity (R/G)
Cell Growth and Death	Apoptosis	nuclear factor of kappa light polypeptide gene enhancer in B-cell	0.6326
Lipid Metabolism	Sterol biosynthesis	phosphomevalonate kinase	0.6359
Metabolism of Cofactors and Vitamins	Ubiquinone biosynthesis	protein geranylgeranyltransferase type I, beta subunit	0.5564
Metabolism of Complex Carbohydrates	N-Glycans biosynthesis	protein geranylgeranyltransferase type I, beta subunit	0.5564
Translation	Ribosome	ribosomal protein L13a	0.6577

3. Comparisons between 2-D PAGE and cDNA microarray results

We compare the proteins that regulated by IL-1β (22 proteins) in 2-D PAGE with genes, which are included in the probe list (11 proteins) in cDNA microarray data. The comparison of cDNA microarray and proteomic analytical results showed large discrepancies between mRNA and protein levels. There were no matched data between proteomic analysis and cDNA microarray analysis (Table 14). This indicates that those proteins may be regulated by post-transcriptional regulation but, not by transcriptional activity.

Table 14. Data comparison between 2-DE PAGE and cDNA microarray

Regulated Proteins	6 hours treatment		24 hours treatment		Pathway	Functions
	2-DE	Microarray	2-DE	Microarray		
HSP90-beta	Up	Unchaged	Up	Unchanged	-*	Molecular chaperone, cell cycle control and signal transduction
Similar to tubulin alpha 2	Up	Unchanged	Up	Unchanged	-	Structural protein
Alpha enolase	Up	Unchanged	Up	Unchanged	Carbohydrate and amino acid metabolism	DNA binding protein, transcription factor, transcription co-repressor (c-myc)
Glutathione S-transferase	Down	Unchanged or Up	Down	Unchanged or Up	Metabolism of other amino acids	Cellular defense against toxic, carcinogenic, and pharmacologically active electrophilic compounds
Malate dehydrogenase, cytosolic	Down	Unchanged	Down	Unchanged	Amino acid metabolism	Cellular metabolism, involve in TCA cycle
Inorganic pyrophosphatase	PTM* (MW*↑)	Unchanged	PTM (MW↑)	-	Energy metabolism	regulator of responses to stresses and adjustments for survival
Cytokeratin 1	PTM (MW↓)	Unchanged	PTM (MW↓)	Unchanged	-	Structural protein
Calponin 3	PTM (MW↓)	Unchanged	PTM (MW↓)	Unchanged	-	Regulation and modulation of smooth muscle contraction, actin-binding protein
HSP27	PTM (MW↓)	Unchanged	PTM (MW↓)	Unchanged	-	Stress resistance and actin organization
Heterogenous nuclear ribonucleoprotein C1/C2	PTM (MW↓)	-	PTM (MW↓)	-	-	Role in ribonucleosome assembly by neutralizing basic proteins
Cytokeratin 8	PTM(pI change)	Down	PTM (pI change)	Down	-	Structural proteins

* PTM: post-translational modification
* MW: molecular weight
* "-" indicates that this gene is included in the probe list but is not detected.

We summarized the genes encoding mucinous and serous component of airway secretion with cDNA microarray. Among the mucin genes, MUC2 was not detected and MUC4, MUC5B, and MUC9 were down-regulated by induction with IL-1β, but only the sialomucin was up-regulated (Table 15). Among the serous component, lysozyme and secretory leukocyte protease inhibitor (SLPI) was up-regulated by induction with 24-hour IL-1β treatment (Table 15). However MUC5AC was not included in the probe list of this microarray system.

Table 15. cDNA microarry data of proteins in airway secretion

Protein in airway secretion			6 hour-treatment	24 hour-treatment
Mucinous Component	**Mucin Genes**	**MUC2**	-*	-
		MUC4	Down	-
		MUC5B	Down	Down
		MUC9	Down	Down
	CD164 antigen, sialomucin		Up	Up
Serous Component	**Lysozyme**		Down	Up
	Secretory Leukocyte Protease Inhibitor (SLPI)		-	Up

* "-" indicates that this gene is included in the probe list but is not detected.

IV. DISCUSSION

Mucus hypersecretion causes many clinical problems, such as rhinorrhea, sputum, and even death due to respiratory failure. It has been reported that mucin is the major component of mucus and MUC5AC and MUC5B are the major mucins in human airways.[8-11] The mechanism of the regulation of mucin secretion by inflammatory cytokines in the airway is very important, and the understanding of this mechanism may offer new therapeutic strategies for the inhibition of airway mucus hypersecretion.

Recently, we found that the signal transduction pathway of IL-1β- and TNF-α-induced MUC5AC overexpression involved ERK/p38 MAP kinases-MSK1-CREB activation in human airway epithelial cells.[12] However, many proteins and genes other than MAP kinase, MSK1, and CREB can also be involved in IL-1β-induced mucus hypersecretion. High-throughput detection of candidate factors that regulate mucus hypersecretion induced by IL-1β has not been reported. Accordingly, we wanted to analyze the effect of the IL-1β on differentially expressed gene and protein profile related to hypersecretion of mucin affected by IL-1β from lung mucoepidermoid carcinoma cell line (NCI-H292 cells), which is widely used in the genetic study of human airway epithelium, using 2-D PAGE with MALDI-TOF MS and cDNA microarray.

Only 22 proteins (1.7%) from 1,300 expressed proteins were found to be regulated by 6- or 24-hour-treatment with IL-1β in 2-D PAGE proteomic study. In the same experiment with normal human nasal epithelial (NHNE) cells, regulated proteins induced by IL-1β were also little (data not shown). These findings can be explained by the limitation of 2-D PAGE. Much of the regulated proteins may either have extremely high molecular weight over 120 kDa, extremely low molecular weight under 10 kDa, low copy proteins, basic proteins which pI is over 9, or

hydrophobic proteins such as membrane proteins. Secondly, there might be earlier and transient change of proteins by IL-1β before the sample collection. Lastly, thorough cell surface washing before sample collection may have removed secreted proteins from the cell surface and therefore may deteriorate the results by excluding the effect of the degree of *de novo* synthesis and extent of degranulation/exocytosis.

Among the up-regulated proteins in 2-D PAGE results, heat shock protein (HSP) is the only protein that may be related to mucus secretion. Although the effect of HSP on mucus secretion has not been fully elucidated, microbial HSP is known to have anti-inflammatory function and suppress goblet cell hyperplasia and mucin hyperproduction,[13] which mechanism is mediated by T lymphocyte in *in vivo*. HSPs may contribute to mucosal defense mechanisms and mucosal healing, most probably through protecting key enzymes related to cytoprotection.[14] The roles of up-regulated HSP 90 are thought to be the compensatory mechanism to control the hypersecretion of mucin and mucosal protective function induced by IL-1β. However, the effect of HSP on mucus hypersecretion remains to be solved.

Genes in microarray data can be divided into two categories, metabolic and regulatory pathways according to the function of those genes.[15] Metabolic pathway affects the new phenotype and regulatory pathway governs the adaptive response[15] of cells. In this cDNA microarray data, most of the regulated genes of 6- or 24-hour-treatment with IL-1β were mainly in the metabolic pathway (6 hr – 73.0%, 24 hr – 63.8%) rather than regulatory pathway (6 hr – 27.0%, 24 hr – 36.2%). According to the previous study, IL-1β, however, did not induce the phenotypic change of airway epithelium.[6,16] It suggests that regulatory genes may play more important roles in mucus hypersecretion than metabolic genes, although the portion of regulatory genes is smaller compared to that of metabolic genes.

The comparison of cDNA microarray and 2-D PAGE analytical results showed large discrepancies (Table 14) between mRNA and protein expression levels like other reports.[17-20] There were changes of protein expression level without any changes of gene level. These suggest that the regulated proteins might be modulated by post-transcriptional regulations, not by transcriptional activities. These discrepancies can be explained by use of a different method of assay/sample preparation, different detection sensitivity, translational regulation (modulation of translational activity), alternative splicing of certain mRNA, post-translational modification, selective degradation or excretion of proteins and time discrepancy between the gene expression and protein expression. Unchanged transcript abundance in the face of regulated protein levels indicates post-transcriptional regulation following IL-1β treatment. Unchanged transcripts in the face of regulated protein level of the corresponding proteins may indicate previous transcription of these genes in an earlier period of the IL-1β treatment of the NCI-H292 cells, producing stable protein species that have undergone post-translational alteration following IL-1β treatment. Taken together, these suggest that mRNA transcript levels provide little predictive value with the extent of protein expression.

We analyzed the several genes that encode the important secreted proteins comprising airway mucus component from the microarray data (Table 15). Interestingly, MUC5B, a major mucin gene[10] of the airway, was down-regulated after 6- or 24-hour treatment with IL-1β. This finding was in accordance with our previous report that IL-1β does not induce MUC5B mRNA expression.[21] These suggest that MUC5B may not be a major mucin induced by IL-1β. Sialomucin CD 164, known as membrane glycoprotein, was up-regulated after both 6- and 24-hour treatment with IL-1β. Sialomucin induced by IL-1β can make the airway secretions more acidic and viscous, which induces mucosal damage, airway obstruction, and secondary inflammation caused by viscous mucus plug.[22] These suggest that increase in sialomucin induced by IL-1β may play a critical role in mucus hypersecretiona and

airway inflammation. Secretory leukocyte protease inhibitor (SLPI) and lysozyme, which are serous secretions in the airway, were also up-regulated by IL-1β as described earlier.[23,24] These suggest that IL-1β induces serous secretion as well as mucous secretion.

The final goal for the description of a biological system should not only use the analysis of mRNA transcript levels alone but also the concurrent analysis of protein expression levels and their functional activities. Although we used both cDNA microarray and 2-D PAGE proteomics, they may have some limitations in providing complete information of the mechanism by which the cell is regulated because of the quite different scope of evaluating fields between the two methods.

V. CONCLUSION

HSP and sialomucin may be closely related to mucus hypersecretion, and their functional validation should be addressed. However, there was poor correlation between mRNA expression and protein abundance in the analysis using 2-D PAGE and cDNA microarray. Due to the quite different scopes of evaluating fields between genomics and proteomics, they may not provide complete information on the gene and protein profiles.

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Abstract (in Korean)

Interleukin 1-β 를 처치한 NCI-H292 세포에서의 유전자 및 프로테옴 분석

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지 장 훈

점액 과분비는 인체기도의 염증성 질환을 야기하는 주원인이다. 저자들은 다방면의 염증반응을 일으키는 사이토카인인 interleukin-1β 가 점액소(MUC2, MUC5AC, MUC8)를 증가시킴으로써 기도의 염증에 중요한 역할을 하고 있음을 밝힌 바 있다. 하지만 아직도 기도의 점액 과분비를 일으키는 분자생물학적 기전은 잘 알려져 있지 않다. 기도의 점액과분비를 일으키는 기전을 이해하기 위해 저자는 IL-1β 를 6 시간 또는 24 시간 처치한 폐의 점액상피양암종 세포주 (NCI-H292 cells)를 가지고 2-dimensional polyacrylamide gel electrophoresis (2-D PAGE)와 cDNA microarray (8.6K)를 이용하여 차별적으로 발현되는 단백 및 유전자를 분석하였다.

2-D PAGE 에서 저자는 IL-1β 의 처치에 의해 차별적으로 발현되는 8 개의 단백과 post-translational modification 을 보이는 14 개의 단백을 발견할 수 있었다. cDNA microarray 를 통해서는 6 시간 처치에서는 413 유전자 (6.6%), 24 시간 처치에는 115 유전자 (2.0%)가 각각 조절되었다. IL-1β 에 의해 조절 발현된 유전자는 유전자의 기능상 분류 중 대사성 유전자에 속하는 경우가 (6 시간 처치군- 73.0%, 24 시간 처치군- 63.8%) 조절성 유전자에 속하는 경우보다 (6 시간 처치군- 27.0%, 24 시간 처치

군- 36.2%) 많았다. 프로테옴 분석과 microarray 분석을 비교해 보면, 유전자의 발현과 단백질의 발현 사이에서 상당한 불일치를 관찰할 수 있었다. 기도에 분비되는 단백질에 해당하는 유전자를 microarray 를 통해 분석해 보면, MUC5B 는 감소하는 반면에 sialomucin CD 164 와 lysozyme, secretory leukocyte protease inhibitor (SLPI)는 증가함을 관찰할 수 있었다. 본 연구를 통하여 유전자 전사양만으로는 단백질 발현의 양을 예견하기는 힘들며, 유전자적 연구방법과 단백질적 연구방법의 분석분야가 상당히 다르기 때문에 이들이 유전자 및 단백질의 완전한 정보를 제공하기는 어려울 것으로 사료된다.

핵심되는 말: 2-dimensional polyacrylamide gel electrophoresis, cDNA microarray, 점액, 과분비