

Interleukin-1 β 를 처리한 기도상피세포의 프로테오믹 및 트랜스크립토믹의 분석

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Proteomic and Transcriptomic Analysis of Interleukin-1 β Treated Airway Epithelial Cells

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ABSTRACT

Background and Objectives : Mucin hypersecretion is one of the main symptoms of inflammatory diseases in the respiratory tract. The authors previously reported that pleiotypic pro-inflammatory cytokine, interleukin (IL)-1 β , plays significant roles in the respiratory tract inflammation by inducing mucins (MUC2, MUC5AC, MUC8). However, the molecular mechanism for mucin hypersecretion in the respiratory tract is still unclear. **Materials and Method** : In order to understand the mechanisms of mucin hypersecretion in the airway epithelium, the differentially expressed proteins and genes in the lung mucoepidermoid carcinoma cell line (NCI-H292 cells), which were treated for 6 and 24 hours with IL-1 β (10 ng/ml), were identified using 2-dimensional polyacrylamide gel electrophoresis (2-D PAGE) proteomics and cDNA microarray analysis (8.6 K). **Results** : In the 2-D PAGE, 8 differentially expressed proteins and 14 post-translational modification proteins were identified 6 and 24 hrs after the IL-1 β treatment. Microarray analysis identified a total of 413 genes (6.6%) in the 6-hour treatment group and 115 genes (2.0%) in the 24-hour treatment group that were regulated after the IL-1 β treatment. The differentially expressed genes that were regulated by the IL-1 β treatment were mostly found in the metabolic pathway rather than in the regulatory pathway. A comparison of the proteomic and microarray data showed that there was a large discrepancy between the protein expression and the gene expression levels. Among the genes encoding the proteins secreted in the airway, MUC5B was down-regulated but sialomucin CD 164, lysozyme, and the secretory leukocyte protease inhibitor (SLPI) were up-regulated. **Conclusion** : These results clearly show that the transcript levels have little value in predicting the extent of protein expression. Genomics and proteomics have different evaluation fields. Therefore, they may not provide all the information on the gene and protein profiles. (Korean J Otolaryngol 2005;48: 158-71)

KEY WORDS : 2-Dimensional polyacrylamide gel electrophoresis · cDNA microarray · Mucus · Mucin · Hypersecretion.

pattern
가

1-4) (mu-
cin)

: 2004 4 30 / : 2004 7 13
: , 120 - 752 134

Interleukin(IL) - 1
(COPD),
(cystic fibrosis)

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⁵⁾ IL - 1

MUC8) 가
IL - 1
IL - 1
2 - D PAGE(2 - dimensional polyacrylamide gel electrophoresis) MALDI - TOF (matrix - assisted laser desorption/ionization - time of flight) MS(mass spectrometry) cDNA microarray
Complete protease inhibitor cocktail tablets(Roche Applied Science, Indianapolis, IN, USA), Pharmalyte 3 - 10(Pharmacia Corporation, Peapack, NJ, USA) trypsin(Promega Corporation, Madison, WI, USA).
Sigma - Aldrich(Saint Louis, MO, USA)
EttanDalt system Hoefer(San Francisco, CA, USA)
isoelectric focusing(IEF), Multiphor, Immobiline DryStrip Kit, Immobiline DryStrips(24 cm, pH 3 - 10 NL ; nonlinear), IPG buffer 3 - 10 NL, DryStrip cover fluid Amersham Biosciences(Piscataway, NJ, USA)

(lung mucoepidermoid carcinoma) (NCI - H292) American Type Culture Collection(CRL - 1848 ; Manassas, VA, USA)
10% fetal bovine serum(FBS) 가
RPMI - 1640(GIBCO BRL, Rockville, MD, USA)
37 2 ,
10 ng/ml IL - 1 0, 6, 24
phosphate - buffered saline(PBS)

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

시료 준비

- 80

400 µl lysis buffer(7 M urea, 2 M thiourea, 2% CHAPS, 2% Pharmalyte pH 3 - 10, 100 mM DTE)
4 12,000 × g
Bradford
, 1 mg - 80

2-D PAGE

lysis buffer 450 µl
240 mm, immobilized pH gradient(IPG) strips(pH 3 - 10, nonlinear, IPG Drystrips, Amersham Biosciences, Piscataway, NJ, USA) rehydration
10 가 . Rehydration isoelectric focusing(IEF, Multiphor, Amersham Biosciences, Piscataway, NJ, USA) 100 V , 300 V
, 600 V , 1,000 V , 2,000 V
, 3500 V 22 75000
VHr가 . IEF가 strip 6 M urea, 20% glycerol, 2% SDS, 0.01% bromophenol blue(BPB), 10 mM tributyl phosphine(TBP) equi-
libration . SDS - PAGE 10~18% T, gra-
dient EttanDalt system . PAGE
20 2 W/gel Coomassie
G - 250(Bio - Rad, Hercules, CA, USA)

Protein visualization and software analysis

GS - 800 Calibrated Densimeter(Bio - Rad, Hercules, CA, USA) , Mellanie (SIB, Sweden)
(3 가 0.33
).

질량 분석을 위한 시료의 준비

, 40% methanol, 100% ace-
tonitrile(ACN), 50 mM ammonium bicarbonate(ABC, pH 8.0) 1~3 , 10 vacuum eva-
porator 15 . 0.1~0.2 µg
Promega modified trypsin 가

ABC buffer 가
37 가 MALDI - TOF

POROS R2 column(Applied Biosystems, Foster city, CA, USA)
가 . Column 5% formic acid 100% ACN

기도상피세포의 프로테옴 및 트랜스크립토믹의 분석

R2 column 2 μ l -
 cyano - 4 - hydroxycinnamic acid(CHCA) elution
 MALDI plate(96 \times 2, Applied Biosystems, Foster
 city, CA, USA)

Mass spectrometry

MALDI - TOF Applied Biosystems Vo-
 yager DE - PRO spectrometer(Applied Biosystems, For-
 ster city, USA) 가
 (trypsin autolysis) (internal calibra-
 tion) (842.51 Da, 2211.11 Da).

SWISS - PROT(<http://kr.expasy.org/>) National Cen-
 ter for Biotechnology Information(<http://www.ncbi.nlm.nih.gov>)

2

cDNA microarray

Labeled cDNA의 합성과 hybridization

Labeled cDNA 50 μ g RNA
 RNA IL - 1 NCI - H292 2
 Trizol (MRC, Cincinnati, OH, USA)
 RNA 2 μ l oligo dT(18 mer) primer(1
 μ g/ μ l) 가 , 70 10
 (denature) 2
 가 (reverse transcription)

: 6 μ l First Strand Buffer 5 \times (250 mM Tris - HCl
 pH 8.3, 375 mM KCl, 15 mM MgCl₂)(Invitrogen life
 technologies, Carlsbad, CA, USA), 3 μ l DTT 0.1 M
 (Invitrogen life technologies), 100 μ M dATP(Amer-
 sham Biosciences, Piscataway, NJ, USA), 100 μ M
 dGTP(Amersham Biosciences), 100 μ M dTTP(Amer-

sham Biosciences), 100 μ M dCTP(Amersham Bios-
 ciences), 3 mM Cyanine 3 - dCTP(NEN Life Science
 Products, Boston, MA, USA)() 3 mM Cya-
 nine 5 - dCTP(NEN Life Science Products)().

400 units SuperScript
TM RNase H - Reverse Transcriptase(Invitrogen life
 technologies) 가 . 42
 . 5 μ l stop solution(0.5 M
 NaOH, 50 mM EDTA) 65 10
 (heat inactivation) . Labeled cDNA
 probe (alcohol precipitation)

Hybridization cDNA chip(8.6 K genes, GenoCheck,
 Ansan, Korea)

custom slide cassette 62
 Array 2 washing buffer 1(2 \times SSC, 0.1% SDS)
 , 3 washing buffer 2(1 \times SSC) , 2 wa-
 shing buffer 3(0.2 \times SSC) . Cy3 Cy5
 signal 1.5 가 gene spot IL - 1

2

2 - D PAGE

1300 spots pH 3.0~10.0
 (Fig. 1). IL - 1 가 4
 , 4
 (post - translational modification)가 14
 spots 1.7%
 IL - 1

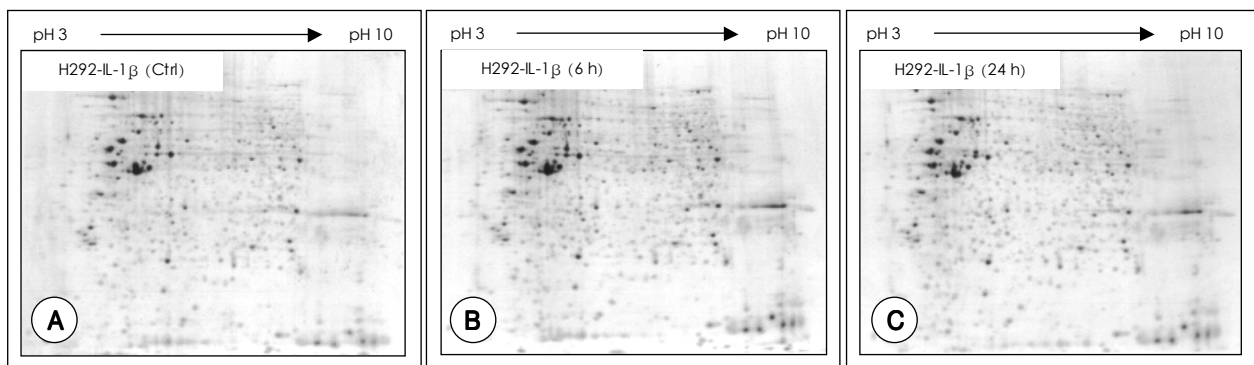


Fig. 1. Master image of 2-D PAGE gel stained with coomassie blue G250. A : 2-D PAGE gel of the control (untreated) NCI-H292 cells. B : 2-D PAGE gel of the 6-hour IL-1 -treated NCI-H292 cells. C : 2-D PAGE gel of 24-hour IL-1 -treated NCI-H292 cells.

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

Protein name	Accession number	Mr (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 alpha-enolase ; carbonate dehydratase	693933	47.109	7.0	1.19E+04	21%

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

Proften name	Accession number	Mr (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%

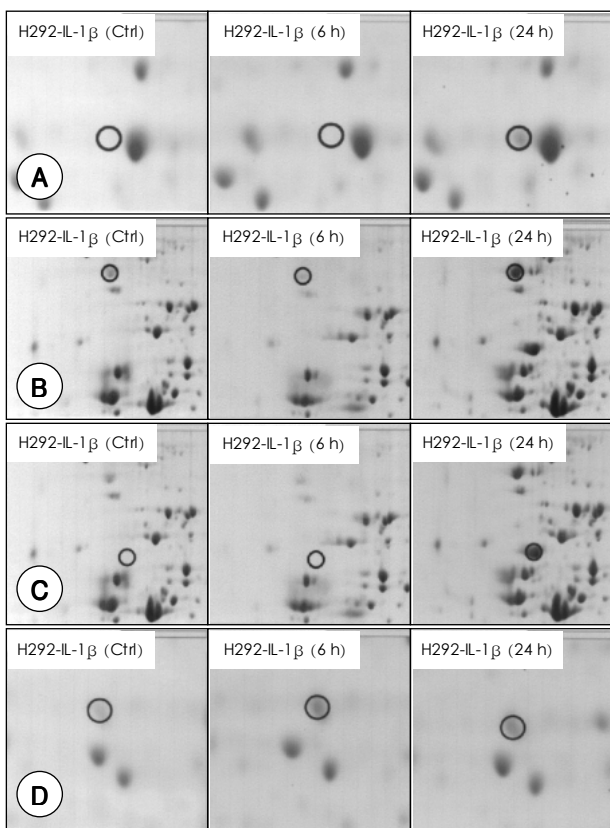


Fig. 2. 2-DE images of up-regulated proteins induced by IL-1 in NCI-H292 cells. A : 2-phosphopyruvate-hydratase alpha-enolase. B : Heat shock protein 90-beta. C : Similar to tubulin alpha 2. D : Alpha enolase.

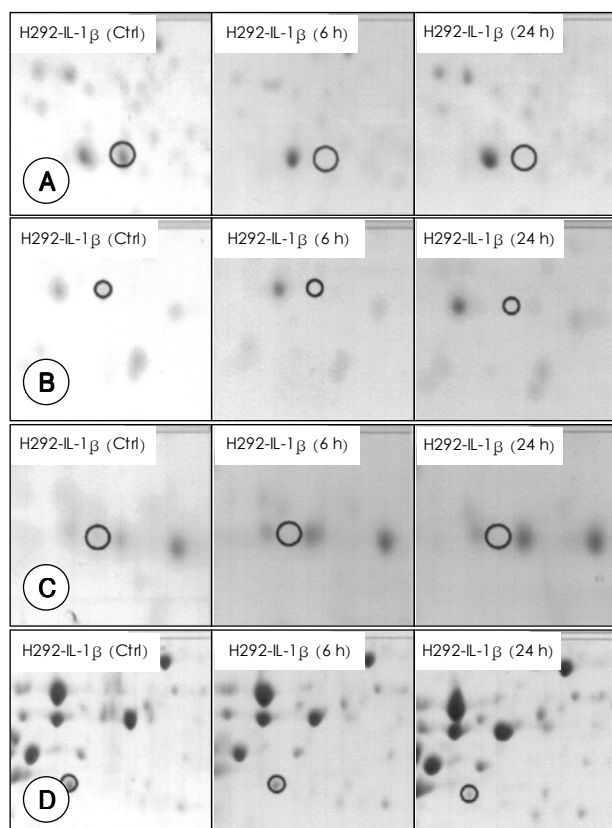


Fig. 3. 2-DE images of down-regulated proteins induced by IL-1 in NCI-H292 cells. A : Glutathione S-transferase. B : Probable thioredoxin peroxidase 1. C : Malate dehydrogenase, cytosolic. D : Unnamed protein product.

발현이 증가된 단백질

가 enolase, the heat shock protein 90 beta, tubulin alpha 2, and 2 - phosphopyruvate - hydratase alpha - enolase(Table 1, Fig. 2)가 .

발현이 감소된 단백질

glutathione S - transferase, thioredoxin peroxidase 1, malate dehydrogenase un-named product(Table 2, Fig. 3)가 .

합성후 변이된 단백질

가 spot pI가 .

분자량이 증가한 단백질

가 inorganic pyrophosphatase erythrocyte cytosolic protein (Table 3, Fig. 4).

분자량이 감소한 단백질

heterogenous nuclear ri-

bonucleoproteins C1/C2, eukaryotic 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 cytoplasmic antiproteinase 3가 (Table 4, Fig. 5).

pI가 변화한 단백질

pI가 cytotkeratin 8, annexin A8 P43

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

Protien name	Accession number	Mr (kDa)	pI	Mowse score	%Coverage
Inorganic pyrophosphatase (PPase)	Q15181	32.660	5.5	2.26E+04	23%
Erythrocyte cytosolic protein (Reptin52)	5730023	51.157	5.5	7.16E+06	37%

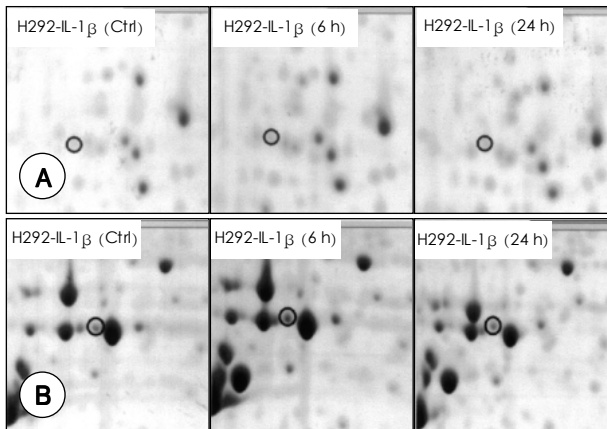


Fig. 4. Post-translational modification of proteins induced by IL-1 in NCI-H292 cells : increased molecular weight. A : inorganic pyrophosphatase. B : erythrocyte cytosolic protein.

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

Protien name	Accession number	Mr (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%

(Table 5, Fig. 6).

cDNA microarray

IL - 1 6 cDNA chip
 8672 6230 가
 (71.8%)(Fig. 7A) 413 가(6.6%) 가
 . 24 5729 가
 (66.1%)(Fig. 7B) 115 가(2.0%) 가

IL - 1 6 6230
 152 가 가 (data not shown)
 261 가 (data not shown). 24
 5729 80 가
 가 (data not shown) 35 가
 (data not shown).
 (metabolic or regulatory pathway)
 (Table 6 - 9). 가

2 - D PAGE cDNA microarray

2 - D PAGE 가 22
 cDNA microarray
 11 cDNA microarray
 ray 11
 . cDNA microarray
 mRNA

cDNA microarray

(Table 10).

(transcriptional regulation)

(post - transcriptional re-

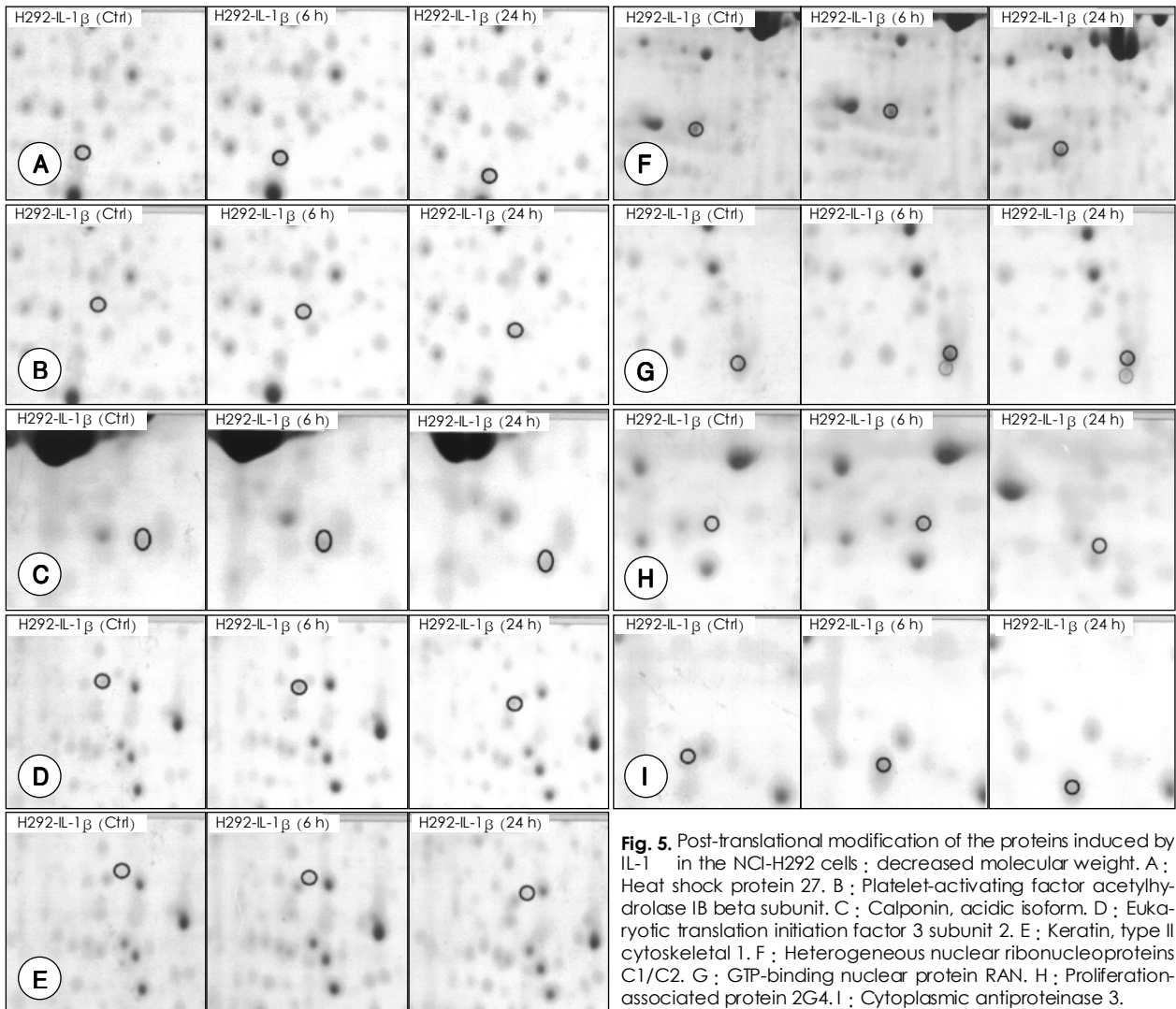


Fig. 5. Post-translational modification of the proteins induced by IL-1 in the NCI-H292 cells : decreased molecular weight. A : Heat shock protein 27. B : Platelet-activating factor acetylhydrolase IB beta subunit. C : Calponin, acidic isoform. D : Eukaryotic translation initiation factor 3 subunit 2. 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 antipeptidase 3.

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

Protein name	Accession number	Mr (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%

gulation)

cDNA microarray (mucinous) (serous) cDNA microarray MUC2 , MUC4, MUC5B, MUC9 , sialomucin 가

(Table 11). , lysozyme secretory leukocyte protease inhibitor(SLPI) IL - 1 24 가 (Table 11). MUC5AC microarray

, 가 (mu-cin) MUC5AC MUC5B (major mucin) 8)9)

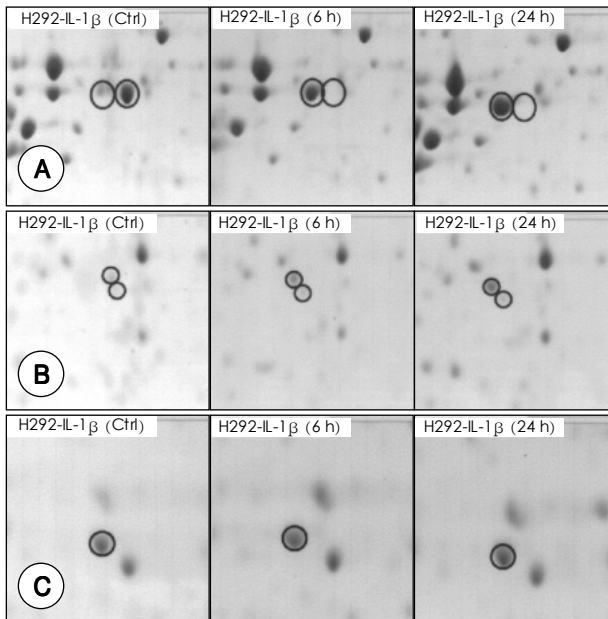


Fig. 6. Post-translational modification of the proteins induced by IL-1β : Change in pl. A : cytokeratin 8. B : annexin VIII. C : P43.

IL - 1 TNF - MUC5AC
 ERK/p38 MAP kinases - MSK1 - CREB¹⁰⁾
 MAP kinase, MSK1, CREB IL - 1
 가
 IL - 1
 high - through-
 IL - 1
 2 - D PAGE proteomic , 1300
 22 (1.7%) IL - 1
 [normal human nasal epithe-
 lial(NHNE) cells] 가 IL - 1
 가 (data not shown).
 2 - D PAGE
 120 kDa 10 kDa

Table 6. Clustering of the up-regulated gene profile of the 6-hour IL-1 treated NCI-H292 cells according to the 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	1.8671
	Benzoate degradation	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, member 2	1.8671
	Glyoxylate and dicarboxylate metabolism	Methylene tetrahydrofolate dehydrogenase	1.7689
	Glyoxylate and dicarboxylate metabolism	Methylene tetrahydrofolate dehydrogenase	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	1.5218
Energy metabolism	Oxidative phosphorylation	NADH dehydrogenase (ubiquinone) Fe-S protein 3	1.7107
Metabolism of cofactors and vitamins	Nicotinate and nicotinamide metabolism	Nucleoside phosphorylase	1.9912
	One carbon pool by folate	Methylene tetrahydrofolate dehydrogenase	1.7689
	Nicotinate and nicotinamide metabolism	Serine threonine kinase 39	1.7244
	Ubiquinone biosynthesis	NADH dehydrogenase 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	1.7244

Table 6. Continued

Main path	Sub path	Name	Intensity (R/G)
Metabolism of complex lipids	Inositol phosphate metabolism	Serine threonine kinase 39	1.7244
	Sphingoglycolipid metabolism	Serine threonine kinase 39	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)	1.7978
	Prion disease	Prion protein (p27 - 30)	1.5895
	Prion disease	Nuclear factor-like 2	1.5450
	Alzheimer's disease	Guanine nucleotide binding 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 polypeptide K	1.5934
	Pyrimidine metabolism	Polymerase (RNA) II polypeptide K	1.5934
	Purine metabolism	Hypoxanthine phosphoribosyltransferase 1	1.5544
	Purine metabolism	Adenylate kinase 2	1.5269
Sorting and degradation	Proteasome	Proteasome subunit, alpha type, 3	1.4972
		Adenylate kinase 3	1.4952
Transcription	RNA polymerase	Polymerase (RNA) II 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

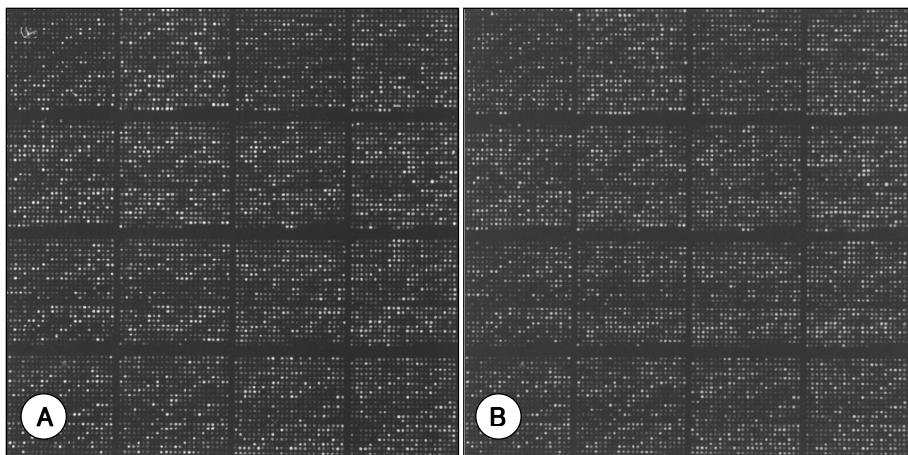


Fig. 7. c-DNA microarray of the IL-1 6-hour-treated (A) and 24-hour-treated (B) NCI-H292 cells. The microarray contains 8672 human genes. Messenger RNA from both control and IL-1 -treated NCI-H292 cells were labeled with either Cy3 or Cy5-dUTP, respectively.

(low copy proteins), pI가 9
(hydrophobic)
2 - D PAGE
가
(earlier and transient change)
de novo synthesis / (degra-
nulation/exocytosis)
2 - D PAGE
가
(earlier and transient change)
let cell)
HSP
HSP
(cytoprotection)
HSP
HSP
(gob-
HSP 90

Table 7. Clustering of the down-regulated gene profile of the 6-hour IL-1 treated NCI-H292 cells according to the 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	0.4920
	Tyrosine metabolism	Aldehyde dehydrogenase 3 family	0.4920
	Phenylalanine metabolism	Aldehyde dehydrogenase 3 family	0.4920
	Glutamate metabolism	Glutamate-ammonia ligase	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	0.5944
	Lysine degradation	Aldehyde dehydrogenase 1 family	0.5944
	Arginine and proline metabolism	Aldehyde dehydrogenase 1 family	0.5944
	Histidine metabolism	Aldehyde dehydrogenase 1 family	0.5944
Tryptophan metabolism	aldehyde dehydrogenase 1 family	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, 70 kDa, polypeptide 2	0.6699
	Benzoate degradation via CoA ligation	Cyclin-dependent kinase (CDC2-like) 10	0.6669
Biosynthesis of secondary metabolites	1, 2-Dichloroethane degradation	Aldehyde dehydrogenase 1 family	0.5944
	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	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	0.5944
	Ascorbate and aldarate metabolism	Aldehyde dehydrogenase 1 family	0.5944
	Pyruvate metabolism	Aldehyde dehydrogenase 1 family	0.5944
	Propanoate metabolism	Aldehyde dehydrogenase 1 family	0.5944
	Butanoate metabolism	Aldehyde dehydrogenase 1 family	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 flavoprotein 1	0.6317
	Nitrogen metabolism	Glutamate-ammonia ligase	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	0.5944
	Bile acid biosynthesis	Aldehyde dehydrogenase 1 family	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, 70 kDa, 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

Table 7. Continued

Main path	Sub path	Name	Intensity (R/G)
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 polypeptide 2	0.6699
	Starch and sucrose metabolism	Cyclin-dependent kinase (CDC2-like) 10	0.6669
	O-Glycans biosynthesis	Sialyltransferase 4C	0.5777
	Peptidoglycan biosynthesis	Glutamate-ammonia ligase	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, 70 kDa, polypeptide 2	0.6699
	Sphingoglycolipid metabolism	Ribosomal protein S6 kinase 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	0.5777
	Sphingoglycolipid metabolism	Arylsulfatase A	0.6210
Metabolism of other amino acids	Glycerolipid metabolism	Aldehyde dehydrogenase 1 family	0.5944
	D-Arginine and D-ornithine metabolism	Glyceraldehyde-3-phosphate dehydrogenase	0.6262
Neuro-degenerative disorders	Beta-Alanine metabolism	Aldehyde dehydrogenase 1 family	0.5944
	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
Nucleotide metabolism	Alzheimer's disease	Amyloid beta (A4) precursor protein	0.6628
	Purine metabolism	Guanylate cyclase 1, soluble, beta 3	0.6338
Signal transduction	Purine metabolism	IMP (inosine monophosphate) dehydrogenase 2	0.5726
	Phosphatidylinositol signaling	Protein tyrosine phosphatase, non-receptor type 21	0.6596
	Phosphatidylinositol signaling	Protein kinase C, zeta	0.5065
	Phosphatidylinositol signaling	Protein kinase C, delta	0.5853
	MAPK signaling pathway	Mitogen-activated protein kinase 10	0.6377
	Phosphatidylinositol signaling	Phosphatidylinositol-4-phosphate 5-kinase, type I, alpha	0.6286
	MAPK signaling pathway	SHC (Src homology 2 domain containing) transforming protein 1	0.5941
Sorting and degradation	MAPK signaling pathway	GRB2-related adaptor protein 2	0.6582
	Ubiquitin mediated proteolysis	F-box and WD-40 domain protein 1B	0.6607
Transcription	Transcription factors	General transcription factor IIF, polypeptide 1, 74 kDa	0.5907
Translation	Ribosome	Ribosomal protein L13a	0.6666
	Ribosome	Ribosomal protein L13a	0.6094

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Table 8. Clustering of the up-regulated gene profile of the 24-hour IL-1 treated NCI-H292 cells according to the 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	TKK protein kinase	1.7002
	Benzoate degradation via CoA ligation	Polo-like kinase (Drosophila)	1.7360
	Benzoate degradation via CoA ligation	TKK 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	1.6441
	Cell cycle	BUB1 budding uninhibited by benzimidazoles 1 homolog	1.6595
	Cell cycle	Polo-like kinase	1.7204
	Cell cycle	MAD2 mitotic arrest deficient-like 1	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	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
	Nicotinate and nicotinamide metabolism	TKK protein kinase	1.7002
	Nicotinate and nicotinamide metabolism	Polo-like kinase (Drosophila)	1.7360
	Nicotinate and nicotinamide metabolism	TKK protein kinase	1.9303
Metabolism of complex carbohydrates	Starch and sucrose metabolism	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	1.6441

Table 8. Continued

Main path	Sub path	Name	Intensity (R/G)
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	TKK protein kinase	1.7002
	Starch and sucrose metabolism	Polo-like kinase (Drosophila)	1.7360
	Starch and sucrose metabolism	TKK 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, 40 kDa	1.5048
	Inositol phosphate metabolism	Inositol (myo)-1 (or 4)-monophosphatase 1	1.6230
	Inositol phosphate metabolism	TKK protein kinase	1.7002
	Sphingoglycolipid metabolism	TKK protein kinase	1.7002
	Inositol phosphate metabolism	Polo-like kinase (Drosophila)	1.7360
	Sphingoglycolipid metabolism	Polo-like kinase (Drosophila)	1.7360
	Inositol phosphate metabolism	TKK protein kinase	1.9303
	Sphingoglycolipid metabolism	TKK 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 9. Clustering of the down-regulated gene profile of the 24-hour IL-1 treated NCI-H292 cells according to the 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

(adaptive response) . cDNA mic-⁶⁾¹³⁾
 roarray IL - 1 가
 (6 - 27.0%,
 24 - 36.2%) cDNA microarray 2 - D PAGE
 (6 - 73.0%, 24 - 63.8%) mRNA 가
 , IL - 1 (Table 10).

가 (transcriptional activity) (post-transcriptional regulation) mRNA alternative splicing, (post-translational modification), 가 mRNA (detection sensitivity), (), mRNA

Table 10. Data comparison between the 2-D PAGE and the cDNA microarray

Regulated proteins	6 hours treatment		24 hours treatment		Pathway	Functions 2-DE
	2-DE	Micro-array	2-DE	Micro-array		
HSP90-beta	Up	Unchanged	Up	Unchanged	- 1)	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 ¹⁾ (Mr ²⁾)	Unchanged	PTM (Mr)	-	Energy metabolism	Regulator of responses to stresses and adjustments for survival
Cytokeratin 1	PTM (Mr)	Unchanged	PTM (Mr)	Unchanged	-	Structural protein
Calponin 3	PTM (Mr)	Unchanged	PTM (Mr)	Unchanged	-	Regulation and modulation of smooth muscle contraction, actin-binding protein
HSP27	PTM (Mr)	Unchanged	PTM (Mr)	Unchanged	-	Stress resistance and actin organization
Heterogenous nuclear ribonucleoproteins C1/C2	PTM (MW)	-	PTM (MW)	-	-	Role in ribonucleosome assembly by neutralizing basic proteins
Cytokeratin 8	PTM (pl change)	Down	PTM (pl change)	Down	-	Structural proteins

1) "-" indicates that this gene is included in the probe list but was not detected, 2) PTM : post-translational modification, 3) Mr : relative molecular mass

Table 11. cDNA microarray data of the proteins in an airway secretion

Protein in airway secretion		6 hour-treatment	24 hour-treatment
Mucinous component	MUC2	-1)	-
	MUC4	Down	-
	MUC5B	Down	Down
	MUC9	Down	Down
	CD164 antigen, sialomucin	Up	Up
Serous component	Lysozyme	Down	Up
	Secretory leukocyte protease inhibitor (SLPI)	-	Up

1) "-" indicates that this gene is included in the probe list but was not detected

microarray

(Table 11). MUC5B IL - 1 6
24

IL - 1 MUC5B mRNA

1 가¹³⁾ MUC5B가 IL -
sialomucin CD 164

IL - 1 6 24 가

IL - 1 sialomucin

(plug)¹⁸⁾

IL - 1 가 sialomucin

secretory leukocyte protease inhi-
bitor(SLPI) (lysozyme) IL - 1
가 IL - 1 가
가
(description of a biological system)

mRNA 가
cDNA microar-
ray 2 - D PAGE 가

HSP sialomucin
가 , mRNA

: 2 - Dimensional polyacrylamide gel electro-
phoresis . cDNA microarray .

2003 (2003 - 02)

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