

Gene Expression Changes Induced by Type IV Allergy-Inducible Chemicals in Dendritic Cells

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ABSTRACT. In the present study, the changes of gene expression profile in dendritic cell (DC)-derived DC2.4 cells sensitized with two allergenic chemicals were analyzed by microarray analysis to develop a basis for an *in vitro* assessment system of type IV allergenic chemicals. Consequently, 26 genes were significantly up-regulated, and 53 were down-regulated in both groups. Interestingly, some of up-regulated genes were associated with the maturation process of DCs. A set of genes was further evaluated by real-time reverse transcription-polymerase chain reaction to identify the gene expression changes specifically induced by type IV allergy-inducible chemicals in DC2.4 cells, and 2 possible candidates, syndecan-1 (Sdc1) and smoothened (SMO) genes were identified. Thus, up-regulation of Sdc1 gene and down-regulation of SMO gene in DC2.4 cells may be diagnostic markers for the screening of type IV-allergenic chemicals.

KEY WORDS: chemical, dendritic cell, microarray analysis, type IV allergy.

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Allergic contact dermatitis (ACD), a type IV allergy, is one of the most common inflammatory diseases of the skin with unknown genetic basis and is often an occupationally related disorder in industrialized countries with an important socio-medical impact [14, 28]. At present, many chemicals are considered to have allergenic potency and thus risk assessment of such chemical substances is important. ACD has been intensively studied, and the development of an allergic hypersensitivity reaction in the skin is considered to be processes depending on the induction of specific T-lymphocyte responses [23]. At the initial step, chemical allergens exposed on the skin are recognized by Langerhans cells (LCs), the principal DC residing in the epidermis and known to play a key role in the development of ACD. Following an encounter with a chemical allergen, LCs are activated and subsequently migrate from the skin to the draining lymph nodes, undergoing a maturation process during the journey [20, 22].

For many years, guinea pigs have been applied for the hazard analysis of skin-sensitizing chemicals [9, 26]. Recently, the local lymph node assay (LLNA) was developed in mice as an alternative approach based on the characterization of initial proliferative responses in draining lymph nodes caused by chemicals exposure [16, 21, 34, 39], and this method is now widely used for estimation of the allergenic potency of chemical substances. Although these approaches are sensitive and reliable, more advantageous ways in terms of cost performance, safety, readiness and animal welfare are expected. More recently, *in vitro* assay for chemical substances with allergenic potency is extensively explored by using cultured cells, especially macroph-

ages [5, 41]. In these experiments, up-regulation of several molecules including cell surface markers were reported to be induced by chemical exposure, suggesting that these markers may be candidates for evaluating chemicals with allergenic potency [5, 41]. However, as well documented, LCs, a family of DCs, are the main antigen presenting cell in the epidermis and have central roles on the induction of allergic skin disorders [4, 38]. In this regard, assessment of LC/DC responses to chemical exposures should be reasonable to know accurate mechanisms of ACD development. Although several studies evaluated the effect of chemicals on DCs by use of *in vitro* differentiated primary DCs, it is disadvantageous because of the limited numbers in the source, donor-to-donor variability and cost for obtaining these cells.

In the present study, the changes of gene expression profile in an established DC line sensitized with allergenic chemicals were analyzed by microarray analysis and real-time reverse transcription-polymerase chain reaction (RT-PCR) to develop a basis for an *in vitro* assessment system of type IV allergy-inducible chemicals.

MATERIALS AND METHODS

Chemicals: Chemical substances, 2,4-dinitrochlorobenzene (DNCB; Wako Pure Chemical Industries, Osaka, Japan), *p*-benzoquinone (BQ; Kanto Chemical, Tokyo, Japan), citral (Cit; Nacalai Tesque, Kyoto, Japan), trimellitic anhydride (TMA; Kanto Chemical) and dextran (Dex; Kanto Chemical) were dissolved in DMSO to a concentration of 10 µg/ml as a stock solution and further diluted with cell culture medium for use. DNCB, BQ and Cit are known to be strong sensitizers and type IV allergy-inducible chemicals [15, 18, 24]. TMA is a respiratory sensitizer and a strong inducer of type I allergy. This chemical is also

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known as an irritant on the skin [10, 17]. Dex is reported to be a non-sensitizer on the skin [7, 39].

Cells: The cell line DC2.4, derived from C57BL/6 mice, was kindly provided by Dr. Kenneth L. Rock (Division of Lymphocyte Biology, Dana Farber Cancer Institute, Boston, MA, U.S.A.). The DC2.4 cells have a dendritic morphology, express DC-specific markers, MHC molecules and co-stimulatory molecules, and have phagocytic activity as well as antigen-presenting capacity [35]. The cells were maintained in DMEM (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% fetal calf serum (FCS; Cansera International Inc., Ontario, Canada), 4 mM L-glutamine and 50 μ M 2-mercaptoethanol at 37°C under 5% CO₂ in air.

Sensitization of DC2.4 cells and RNA extraction: DC2.4 cells were plated at 1.0×10^5 cells/ml and incubated for 12 hr. The cells were treated with chemicals in non-toxic doses (0.1 μ g/ml for DNCB, BQ and Cit, 1.0 μ g/ml for TMA and Dex) and harvested after cultivation for 24 hr. Total RNA was extracted with RNeasy mini kit (QIAGEN K. K., Tokyo, Japan).

Microarray analysis: Total RNA from control and BQ-treated DC2.4 cells was reverse transcribed into cDNA using Fluorescent Direct Label Kit (Invitrogen, Carlsbad, CA). cDNA samples were labeled with Cy3-dCTP or Cy5-dCTP (Amersham Pharmacia Biotech, Piscataway, NJ) and purified with MinElute Mini Kit (QIAGEN K. K.). Microarray was performed with Mouse cDNA Microarray Kit (Agilent Technologies, Palo Alto, CA), and the data was analyzed by Feature Extract software (Agilent Technologies). Microarray analysis of DNCB-treated DC2.4 cells was performed with Filgen Array Mouse 32K kit (Filgen Technologies), and the data was analyzed by GenePix 4000B microarray laser scanner (Axon Instruments, Union City, CA).

Quantification of the gene expression by real time RT-PCR: The real time RT-PCR was performed with the QuantiTect SYBR Green RT-PCR kit (QIAGEN K.K.) in accordance with the manufacture's instructions. Briefly, 25 μ l of a PCR mixture in a tube containing each target gene-specific primer pair at 0.5 μ M, 1 $\times$ QuantiTect SYBR Green RT-PCR Master Mix, 0.25 μ l of QuantiTect RT Mix, and 0.2 μ g of total RNA was subjected to reverse transcription at 50°C for 30 min. After inactivation of reverse transcriptase at 95°C for 15 min, real-time PCR amplification was performed with 40 cycles of denaturation (94°C for 15 sec),

annealing (55°C for 30 sec), and polymerization (72°C for 30 sec). As an internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was assessed by real-time RT-PCR. The target genes and their primer sequences addressed in this study were described in Table 1. For the compensation of accidental errors among samples, relative gene expression numbers were calculated as raw values divided by the amount of GAPDH in the same samples.

RESULTS

Sensitivity of DC2.4 cells to chemical exposures: To determine the dose of chemicals for *in vitro* sensitization to DC-derived DC2.4 cells, the cells were exposed to various concentrations (0–100 μ g/ml) of chemicals for 24 hr, and cell viabilities were determined by trypan blue dye exclusion assay. The viability of DC2.4 cells was largely affected when exposed to DNCB, BQ and Cit at the concentration of 5–10 μ g/ml although their cytotoxicity was low at 1–5 μ g/ml and almost background level at 0–0.5 μ g/ml. On the other hand, the effect of TMA or Dex on the viability of DC2.4 cells was minimal through the examined concentrations (0–100 μ g/ml) (Fig. 1). Based on these findings, we decided to use these chemicals at the concentration of 0.1 μ g/ml on DNCB, BQ and Cit, and 1.0 μ g/ml on TMA and Dex for subsequent experiments.

Microarray analysis of gene expression changes in DC2.4 cells induced by chemical exposures: To determine the typical gene expression changes in DC2.4 cells induced by type IV allergenic chemical exposure, DC2.4 cells were exposed to DNCB or BQ, both of which are known as strong sensitizers of contact hypersensitivity, for 24 hr and subjected to cDNA microarray analysis. In the DNCB-treated DC2.4 cells, 957 genes were up-regulated with fold changes of +1.3 to +4.4 and 1933 genes were down-regulated with fold changes of –1.3 to –9.0 when compared with control samples. On the other hand, in the BQ-treated cells, 790 genes had fold increases in expression over control ranging from +1.3 to +3.9, and 726 genes showed a down-regulation with fold changes of –1.3 to –2.2. When we overlapped these two data, 26 genes were up-regulated, and 53 were down-regulated in both groups. The changed genes represented a wide range of cellular processes including signal transduction, protein modification/synthesis and transcription. A list of genes which were up-regulated in DC2.4 cells

Table 1. Primer sequences used for real-time RT-PCR

Gene name	Gene symbol	Forward primer	Reverse primer
glyceraldehyde-3-phosphate dehydrogenase	GAPDH	5'-TGAACGGGAAGCTACACTGG-3'	5'-TCAGATCCACGACGGACACA-3'
Cytokine inducible SH2-containing protein 2	Socs2	5'-CAGTCAACAGGATGGTACTGG-3'	5'-AGTCTTGTGGTAAAGGCAGTC-3'
Syndecan-1 precursor	Sdc1	5'-TTCATTGTGGGGAGGTCTAC-3'	5'-AAGTTCTCACACAGGCTCTT-3'
Tubulin alpha-6 chain	Tuba6	5'-CTGATGGAGCGGCTCTCTGT-3'	5'-AAGCTGCTCATGGTAGGCTTTC-3'
Lymphocyte antigen 86 precursor (MD-1 protein)	LY86	5'-GGAGAATATCAGCTCTTGCTGG-3'	5'-CCAGGCAATCTCAAGGAAGTG-3'
smoothened	SMO	5'-TTGGATGCAGACTCGGACTT-3'	5'-CCAGAAAAGGGCACTATTGG-3'
Hairy/enhancer-of-split related with YRPW motif 1	Hey1	5'-CTTCGGACATCACCCACACA-3'	5'-GTCCCAACACACCTGGGATT-3'
phosphatidylinositol 3-kinase, C2 domain containing, alpha	Pik3c2a	5'-CCTTTGCTGGGTACATGATGAC-3'	5'-GGAAGGTTAACTGCTCGCTTT-3'
polypeptide epidermal growth factor receptor pathway substrate 8	Eps8	5'-AACTGGCCAGTTTCGGTACTC-3'	5'-GGAGTTGACTTGAAAGGCATGG-3'
exonuclease 1.	Exo1	5'-GCTGGCTGAAGATGACCTGTT-3'	5'-AGCTCAGATTGTGCATCCCAT-3'
N-acylsphingosine amidohydrolase 2; neutral/alkaline; neutral/alkaline ceramid	Asah2	5'-AACAAACCATGTTCGGACGAA-3'	5'-GAGGAAGGTTTGATGGGTCTGG-3'
carboxypeptidase D	Cpd	5'-CAACTTCACCCCTTGTCGATCT-3'	5'-CAGTTCCAACCTGGAGCGTTT-3'

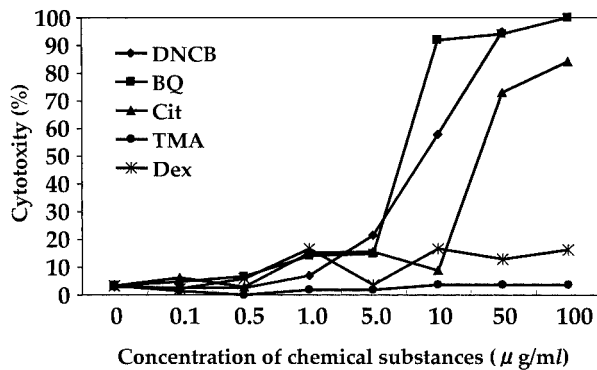


Fig. 1. Sensitivity of DC2.4 cells to chemical exposures. DC2.4 cells were exposed to chemicals at various concentrations (0–100 µg/ml) for 24 hr, and cell viabilities were determined by trypan blue dye exclusion assay.

after exposure to DNCB or BQ for 24 hr are presented in Table 2 along with their accession number and fold-change relative to the control cell cultures. The genes down-regulated are also shown in Table 3.

Real-time RT-PCR analyses of gene expression changes in DC2.4 cells induced by chemical exposures: In order to determine the reproducibility of the gene expression changes observed in microarray analysis, a set of genes was selected for evaluation by real-time RT-PCR. In the previous study, we assessed *in vivo* changes of gene expression in mouse ear sensitized with a type IV allergy-inducible chemical, BQ (data not shown). The microarray data from DNCB or BQ-treated DC2.4 cells were compared with that from type IV chemical-exposed mouse ears, and up- or down-regulated genes in all the experiments were explored. As the result, 3 up-regulated (Socs2, Sdc1 and Tuba6) and 8 down-regulated (Eps8, Exo1, Asah2, Cpd, LY86, SMO, Hey1 and Pik3c2a) genes showed strict changes in all the experiments,

Table 2. List of genes that were up-regulated by 24 hr exposure to DNCB and BQ

Accession no.	Gene name	Gene symbol	Fold-change	
			DNCB	BQ
Cytokine				
M27960	Interleukin-4 receptor alpha chain	Il4ra	1.3	1.5
AK078708	LPS-induced TNF-alpha factor	Litaf	1.6	1.3
Protein modification/synthesis				
AJ578468	defensin beta 14	NM_183026	1.7	1.4
U80019	proline dehydrogenase (oxidase) 2	Prodh2	1.3	1.4
AK054050	amine oxidase (flavin-containing)	Maob	1.3	1.5
L07645	Histidine ammonia-lyase	Hal	1.5	1.3
AK007058	L-lactate dehydrogenase C chain	Ldh3	1.8	2.7
AI323038	Cathelin-related antimicrobial peptide	Camp	1.4	1.4
NM_008185	Glutathione S-transferase theta 1	Gstt1	1.6	1.4
AK076002	proteasome 26S non-ATPase subunit 11	Psmd11	1.7	2.4
Signal transduction				
AK036928	MAP kinase kinase 4	Map2k4	1.5	1.7
U88327	Cytokine inducible SH2-containing protein 2	Socs2	1.7	1.3
AK122347	Dedicator of cytokinesis protein 10	Dock10	2.7	1.5
Cell surface receptors/membrane proteins				
AF237914	membrane-spanning 4-domains.	Ms4a4c	1.7	1.4
NM_011519	Syndecan-1 precursor	Sdc1	1.6	1.3
BC057133	Chloride channel protein 3	Clcn3	1.4	1.4
Transcription factors/activators/repressors				
L09600	nuclear factor, erythroid derived 2	Nfe2	1.4	1.7
AK131183	Nuclear factor erythroid 2 related factor 1	Nfe2l1	1.7	1.7
Cell cycle/proliferation/death				
K014411	Synaptonemal complex protein 2	SCP2	1.6	1.5
Others				
BC040397	3 beta-hydroxysteroid dehydrogenase	Hsd3b2	2.1	1.9
U12147	Laminin alpha-2 chain	Lama2	1.4	1.4
AK081350	phosphatidylinositol 3-kinase, catalytic.	Pik3cb	2.3	1.7
M13441	Tubulin alpha-6 chain	Tuba6	1.3	1.6
BC010745	tubulointerstitial nephritis antigen	Tinag	1.4	1.7
L29468	Cofilin, muscle isoform (Cofilin 2)	Cfl2	1.6	1.3
XM 354745	Proliferin 1 precursor	Mrpplf3	1.3	1.4

Table 3. List of genes that were down-regulated by 24 hr exposure to DNCB and BQ

Accession no.	Gene name	Gene symbol	Fold-change	
			DNCB	BQ
Cell surface receptor/membrane proteins				
Y18365	CD97 antigen precursor.	Cd97	-1.6	-1.7
K027929	Lymphocyte antigen 86 precursor	LY86	-2.2	-2.1
K014556	epidermal growth factor receptor pathway	Eps811	-1.4	-1.6
AJ251594	CD44 antigen	CD44	-1.3	-1.3
F263247	macrophage erythroblast attacher	Maea	-1.4	-1.3
F033017	potassium channel. subfamily K. member 1	Kcnk1	-1.4	-1.6
F089721	smoothened	SMO	-1.4	-2.4
Transcription factors/activators/repressors				
AF172286	Hairy/enhancer-of-split related with YRPW	Hey1	-1.8	-1.8
F004295	myelin transcription factor 1-like	Myt11	-1.4	-1.8
BC048845	inhibin beta-B	Inhbb	-1.4	-1.4
BC052697	splicing factor 3a. subunit 2	S3A2	-1.4	-1.6
BC068268	POU domain. class 5. transcription factor 1	Pou5f1	-1.4	-1.5
BC057126	Cbp/p300-interacting transactivator	Cited2	-1.3	-1.5
Y10926	binding protein suppressor of hairless-like	Rbpsuhl	-1.4	-1.6
AK049260	RNA polymerase II transcriptional coactivator	Rpo2tc1	-1.4	-1.5
U83630	nuclear antigen Sp100	Sp100	-1.4	-1.4
AK031025	enhancer of polycomb homolog 2	Epc2	-1.4	-1.4
Cell cycle/proliferation/death				
Z26580	Cyclin A2 (Cyclin A).	Ccna2	-1.6	-1.8
AK004355	cyclin-dependent kinase 2-interacting protein.	NM_026048	-1.8	-1.5
AF060246	zinc finger protein 106	Zfp106	-1.3	-1.3
AF316548	zinc finger protein 148	Zfp148	-1.4	-1.5
AF068780	geminin	GEMI	-1.4	-1.3
BC065144	meiotic recombination 11 homolog A	Mre11a	-1.3	-1.5
U52193	phosphatidylinositol 3-kinase. C2	Pik3c2a	-1.4	-1.8
Small molecule transport				
AK015400	ferritin heavy chain 3; mitochondrial ferritin.	NM_026286	-1.6	-1.6

and thus these genes were subjected to further real-time RT-PCR analysis. The real-time RT-PCR results showed that all up-regulated genes and 6 of 8 down-regulated ones corresponded to microarray analysis data although Sdc1 and Pik3c2a showed only limited changes when compared with microarray analysis data (Fig. 2).

To determine whether any of the significant gene changes observed were due to the reactive nature of the contact allergen, DC2.4 cells were exposed to 1.0 $\mu\text{g/ml}$ DMSO (solvent used to dissolve chemicals as a control), 0.1 $\mu\text{g/ml}$ BQ (a type IV allergy-inducible chemical), 0.1 $\mu\text{g/ml}$ Cit (a type IV allergy-inducible chemical), 1.0 $\mu\text{g/ml}$ TMA (an irritant on the skin and a type I allergy inducer) and 1.0 $\mu\text{g/ml}$ Dex (a non-sensitizer on the skin) for 24 hr, and gene expression changes were determined by real-time RT-PCR. As shown in Fig. 2, all 3 up-regulated genes chosen were enhanced, and the 8 down-regulated genes were diminished their expression by BQ- or Cit-treatment for 24 hr although some genes such as Tuba6 and Cpd showed limited changes. When treated with TMA, enhanced expression of LY86 and SMO genes, which are down-regulated after type IV allergy-inducible chemicals, was observed (Fig. 2). Socs2 gene, which was up-regulated after sensitization by type IV

allergy-inducible chemicals, was also up-regulated by the TMA-treatment, while the expression levels of other genes were scarcely affected by TMA (Fig. 2). In case of Dex-treated cells, the changes of gene expression levels were seen on some genes; up-regulation of SMO and down-regulation of Sdc1, Eps8, Exo1, Asah2, Hey1 and Pik3c2a (Fig. 2). From these results, up-regulation of Sdc1 gene and down-regulation of SMO gene in DC2.4 cells may be specific for type IV-allergy inducible chemicals.

DISCUSSION

In the present study, we focused on the induction phase of type IV allergy and attempted to identify the genes specifically changed by type IV allergy-inducible chemicals in DCs. For this purpose, changes of gene expression profile in DCs to hapten exposure were evaluated by microarray analysis, since LCs, a family of DCs, were shown to have central roles on the initiation of immune responses including allergic reactions in the skin [4, 38]. Recently, attempts have been made to develop *in vitro* sensitization tests using DCs derived from peripheral blood mononuclear cells or CD34⁺ hematopoietic progenitor cells purified from cord

Table 3. Continued

Accession no.	Gene name	Gene symbol	Fold-change	
			DNCB	BQ
Signal transduction				
U85021	adenylate cyclase 8	Adcy8	-1.3	-1.4
BC012488	Rho guanine nucleotide exchange	Arhgef1	-1.3	-1.3
Y13346	adenosine A2a receptor	Adora2a	-1.4	-1.4
BC085270	RAB11B. member RAS oncogene family	Rab11b	-1.4	-1.5
L21671	epidermal growth factor receptor pathway	Eps8	-1.3	-1.6
Protein modification/synthesis				
D87521	protein kinase. DNA activated. catalytic	Prkdc	-1.4	-1.5
M95408	PTK2 protein tyrosine kinase 2	Ptk2	-1.4	-1.5
Others				
AF071316	COP9 (constitutive photomorphogenic)	Cops7a	-1.9	-1.5
AJ238213	exonuclease 1.	Exo1	-1.5	-1.7
BC056376	Myotubularin-related protein 1	Mtm1	-1.8	-1.6
AB037181	N-acylsphingosine amidohydrolase 2	Asah2	-1.7	-1.5
U94662	Trk-fused gene.	Tfg	-2.2	-1.6
AF123502	DNA polymerase epsilon. catalytic subunit A	Pole	-1.6	-1.7
BC068143	DNA-directed RNA polymerase III	Polr3b	-1.8	-1.5
M94584	chitinase 3-like 3; eosinophil chemotactic	Chi3l3	-1.9	-2.3
AK078888	interferon-related developmental regulator 1	Ifrd1	-1.4	-1.4
AF045252	tousled-like kinase 2 (Arabidopsis)	Tlk2	-1.3	-1.3
BQ928977	tumor protein D52	Tpd52	-1.3	-1.5
X84692	spermatid perinuclear RNA binding protein	Strbp	-1.3	-1.6
CA478631	metallothionein 1	Mt1	-1.3	-1.5
AF031939	RalBP1 associated Eps domain containing	Reps1	-1.4	-1.5
X97982	poly(rC) binding protein 2	Pcbp2	-1.4	-1.4
D85391	carboxypeptidase D	Cpd	-1.4	-1.8
NM_007622	Chromobox homolog 1 (Drosophila HP1 beta)	Cbx1	-1.4	-1.5
BC056376	myotubularin related protein 1	Mtm1	-1.4	-1.6
AF411253	EF hand calcium binding protein 2	Efcfbp2	-1.4	-1.4
BC079642	abl-interactor 1	Abi1	-1.4	-1.4
BC011246	hemopexin	HEMO_MOUSE	-1.4	-1.3

blood or bone marrow [1, 36]. However, the use of *in vitro* differentiated primary DCs is difficult due to the nature of these cells such as low numbers in the source and donor-to-donor variability [2]. In addition, treatment with several cytokines is generally applied to obtain DCs from blood or bone marrow cells [3, 11, 30], and this process probably changes the cell reactivity to stimulations. Thus, established cell lines are preferable to standardize the condition among assay. A recently established DC line, DC2.4 cells, was applied as a target cell for this assay, and its reactivity to chemical exposures was addressed by microarray analysis. As the result, many gene expression changes were observed after treatment with two different allergenic chemicals, DNCB and BQ. Overall, the changes seemed to be not so noticeable. It is because of the nature of this cell line since the human monocyte-derived THP-1 cells with the same treatment extensively changed a large number of gene expression profile (data not shown). In addition, similar results to our data were reported in a recent study using primary DCs from peripheral blood after chemical treatment [31, 36, 37], suggesting that the effect of sensitization on the gene expression levels might be relatively mild in DC lineage. We analyzed the two data from DNCB- and BQ-sen-

sitized cells and tried to line up the candidate genes specifically up- or down-regulated by type IV allergy-inducible chemicals. As the results, 26 genes were shown to be up-regulated, and 53 were down-regulated in both groups. Interestingly, some of up-regulated genes were associated with the maturation process of DCs. These include TNF- α (a maturation-inducing cytokine), Sdc-1 (a cell surface proteoglycan induced during the maturation process), Map2k4 (a member of MAP kinase kinase family associated with migration and maturation of DC) and Socs2 (a suppressor of cytokine signaling molecule induced during the maturation process) [12, 19, 40]. In addition, up-regulation was also detected on defensin and cathelin, which were formerly considered to work just as antimicrobial peptides [6, 8] and recently reported to have cell migration activity and to be associated with DC maturation process [25]. In contrast, down-regulation of CD44 was detected, which is reported to be expressed on mature DC and induce adhesion with T cell. This may be explained by that the time point at 24 hr after sensitization is still in the process of maturation. In the previous studies, many other molecules are reported to be associated with DC maturation process; for instance, up-regulation of transcripts for the co-stimulatory molecules

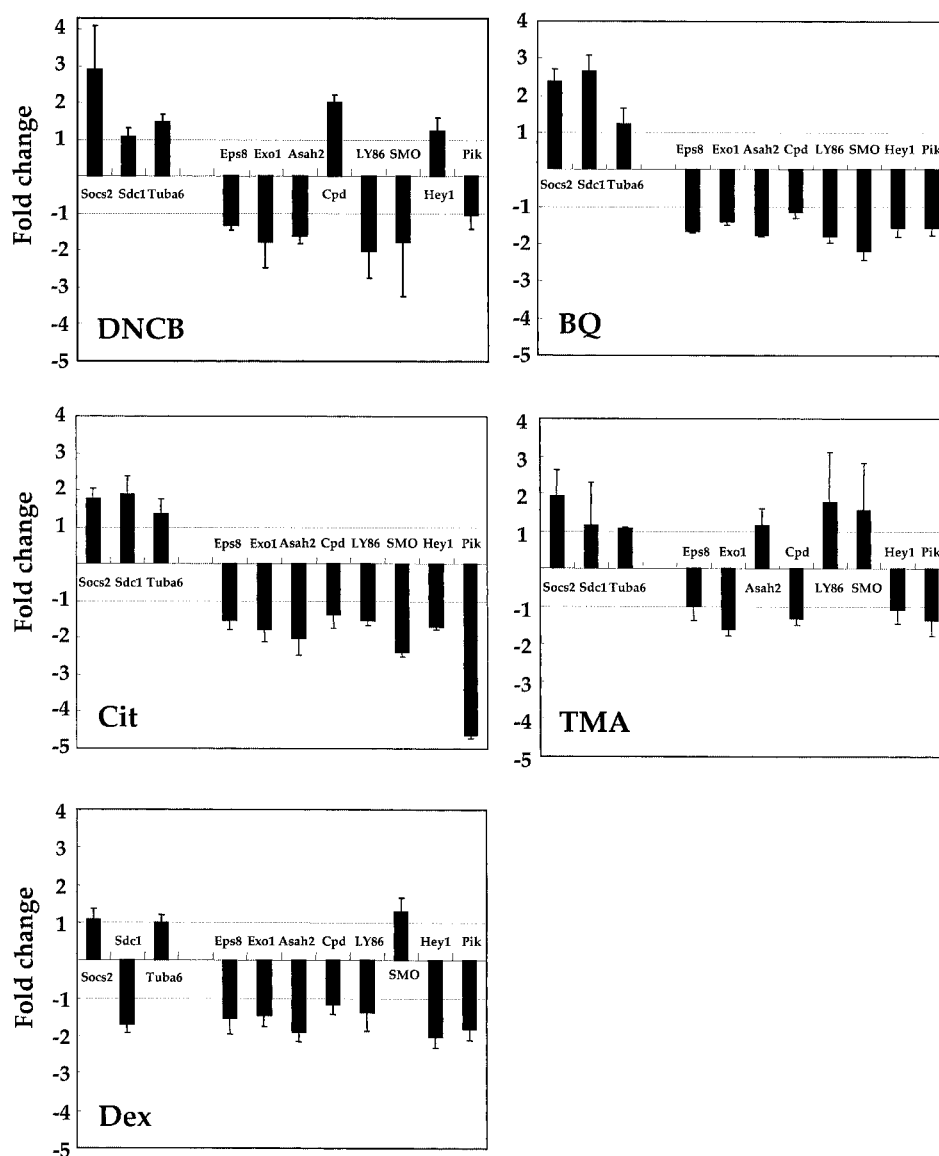


Fig. 2. Gene expression changes in DC2.4 cells induced by DNCB, BQ, Cit, TMA and Dex. DC2.4 cells were exposed to type IV allergy-inducible chemicals, DNCB, BQ or Cit ($0.1 \mu\text{g}/\text{ml}$), a type I allergy-inducible chemical, TMA ($1.0 \mu\text{g}/\text{ml}$) or a non-sensitizer, Dex ($1.0 \mu\text{g}/\text{ml}$) for 24 hr, and the changes of the gene expression were analyzed by real-time RT-PCR. Fold changes were determined based on the gene expression in the cells exposed to solvent DMSO used for solubilization of chemicals.

CD86 [29] and the constitutive chemokine receptor CXCR4 [32, 33], and down-regulation of genes encoding molecules involved in antigen uptake such as the high affinity IgE receptor [27], aquaporin 3 [13]. However, the changes of these molecules were not observed in our study. Assessments of gene expression changes in other time points may detect the up- or down-regulation of these genes. Alternatively, characteristics of DC2.4 cells may give rise to the results.

In order to determine the reproducibility of the gene expression changes observed by microarray analysis, the

data from DNCB or BQ-treated DC2.4 cells were compared with that from type IV chemical-exposed mouse ears (data not shown), and 3 up-regulated (Socs2, Sdc1 and Tuba6) and 8 down-regulated (Eps8, Exo1, Asah2, Cpd, LY86, SMO, Hey1 and Pik3c2a) genes in all the experiments were selected for further evaluation by real-time RT-PCR. DC2.4 cells were treated with TMA, an irritant on the skin and type I allergy inducer, and Dex, a non-hazardous chemical on the skin, in addition to type IV allergy inducers, DNCB, BQ and Cit, to identify contact hypersensitivity-specific changes. Although DNCB-induced up-regulation of Sdc1 gene is

limited, other type IV allergy inducible chemicals, BQ and Cit, markedly up-regulated the gene expression as seen in microarray experiments in DNCB or BQ-exposed DC2.4 cells and DNCB-treated mouse tissues. TMA, an irritant on the skin and type I allergy inducer, also up-regulated Sdc1 gene expression in some experiment; however, the changes are neither significant nor reproducible. Thus, these results suggested that up-regulation of Sdc1 gene and especially, down-regulation of SMO gene in DC2.4 cells correlated with type IV allergic reaction (Fig. 2). In the experiment, Dex-treatment induced expression changes of Sdc1, Eps8, Exo1, Asah2, Hey1 and Pik3c2a genes (Fig. 2). Dex is known as a non-sensitizer on the skin; however, we suspected that it had some stimulatory effects on the cells when sensitized directly. Alternatively, uptake of such a high molecular compound with molecular mass of 60,000–90,000 probably initiated DC activation *in vitro*. At present, the function of the proteins derived from Sdc1 and SMO genes on DCs was not well documented although Sdc1 was shown to be a cell surface proteoglycan induced during the maturation process. Further functional analyses may bring interesting information about the role of these proteins on DC maturation and initiation of type IV allergic reaction.

In conclusion, we tried to identify the gene expression changes specifically induced by type IV allergy-inducible chemicals in DCs by microarray and real-time RT-PCR analyses, and 2 possible candidates, Sdc1 and SMO genes, were identified. Thus, up-regulation of Sdc1 gene and down-regulation of SMO gene in DC2.4 cells may be diagnostic markers for the screening of type IV-allergy inducible chemicals. Further analyses of the genes specifically changed by type IV allergy-inducible chemicals are required to clarify the gene expression profiles. The combination of expression changes on several candidate genes may promise reliable results for screening of the allergic chemicals.

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