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Two-dimensional electrophoresis analyses of atopic dermatitis and the chances to detect new candidate proteins by the variations in immobilized pH gradient strips

Yong-Doo Park^{a,b}, You-Jeong Lyou^b, Jun-Mo Yang^{a,b,*}

^a Department of Dermatology, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul 135-710, Republic of Korea

^b Clinical Research Center, Samsung Biomedical Research Institute, Seoul 135-710, Republic of Korea

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KEYWORDS

2D-PAGE;
IPG strip variation;
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Real-time PCR

Summary

Background: Proteomic approaches, one of the high-throughput technologies, have been used to search for the proteins that are abnormally expressed in human diseases. The atopic dermatitis (AD)-associated genes or proteins are gradually being reported. **Objective:** In accordance with recent reports, we conducted the serial proteomic studies to compare with the protein expression level and to find a critical protein associated with AD.

Methods: We applied two-dimensional electrophoresis (2-DE) coupled with MADI-TOF as well as LC-MS/MS to detect dysregulated protein in the AD proteome obtained from the patient-derived primary cells. Real-time PCR was also conducted to compare with the proteomic results in the transcriptional level.

Results: We successfully detected new AD-associated proteins in the AD-derived fibroblasts 2D-PAGE studies due to the IPG strip variations after conducting MALDI-TOF mass spectrometry and identification of the proteins. From the real-time PCR quantifications, we found that the altered expression of caldesmon 1 isoform 5, nucleophosmin 1, esterase D and chloride intracellular channel 4 were well matched both at the transcriptional and translational levels, and this suggested that these proteins may have important involvement with the pathogenesis of AD.

Conclusion: By simply repeating the trials with changing the commercial strips with different lot numbers in the 2D-PAGE analysis, this provided us with new finding in the

Abbreviations: 2D-PAGE, two-dimensional electrophoresis; IPG, immobilized pH gradient; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; AD, atopic dermatitis; ADe, extrinsic type of AD; ADi, intrinsic type of AD

* Corresponding author at: Department of Dermatology, Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul 135-710, Republic of Korea. Tel.: +82 2 3410 3541; fax: +82 2 3410 3869.

E-mail address: junmo.yang@samsung.com (J.-M. Yang).

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AD-derived samples. This approach we used may increase the chances of finding new candidate proteins in the clinical samples and it may also be applicable for proteomic studies of other diseases.

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1. Introduction

High-throughput based approaches in proteomics and genomics such as 2D-PAGE and DNA-chip microarray have been applied to analyze many diseases and to discover novel disease-specific biomarkers for diagnostic and therapeutic purposes [1,2]. Now more than ever, the rapid developments of techniques in both proteomics and genomics have enabled researchers to understand diseases and diseases-associated factors. With the help of the commercial strips that are currently being marketed, applications of the proteomic approaches, mostly in 2D-PAGE, have evolved to analyze many human diseases and reveal their pathogenesis.

Atopic dermatitis (AD) is a chronic inflammatory skin disease that is accompanied with itchy dry skin, lichenification, pruritic excoriations and flexural dermatitis. The two types of AD have been defined as the extrinsic and intrinsic types. The extrinsic type (ADe) is associated with IgE-mediated sensitization and it accounts for 70–80% of the AD patients, while the intrinsic type (ADi) is not involved with IgE-mediated sensitization and it is seen in 20–30% of AD patients. For the pathogenesis of AD, several cells such as T lymphocytes (Th1 and Th2), Langerhans cells (LCs), eosinophils, and keratinocytes have been regarded as important since these cells are directly related with several blood factors such as cytokines, chemokines and IgE, and they are also related with microbial infection [3–5]. We have recently profiled the differentially expressed protein patterns in AD-derived fibroblasts versus normal fibroblasts by using the proteomic approach [6]. As a result, we have revealed several candidate proteins, including post-translationally modified proteins, which are thought to be involved in the pathogenesis of AD. In a series of 2D-PAGE analyses concerning the AD patient-derived primary cultured skin cells such as fibroblasts [6] and keratinocytes [7], we found that commercial IPG strips have variations that result in different resolutions for the proteome separations.

In this study, we evaluated the new candidate proteins that were found during AD-derived fibroblasts 2D-PAGE studies due to the IPG strip results. From the gel image analyses, MALDI-TOF identification, and real-time PCR quantifications, we sug-

gest several candidate proteins of interest that are thought to be important in AD and these candidates were not detected in our former studies. Our findings can provide useful information regarding the IPG strip results and the pathogenesis of AD.

2. Materials and methods

2.1. Subjects

AD samples were collected from non-asthmatic atopic AD patients according to the methods of our previous reports [8]. We recruited 14 ADe patients, 10 ADi patients and 14 normal specimen donors. The ages of both the control and AD patients were between 19 and 32 years old. The gender of all the sample donors was male. The mean values for each group's total IgE level, eosinophilia count, and SCORAD score were 1546 U/mL, 670 μL^{-1} , and 54.34 for the ADe group and 105 U/mL, 252 μL^{-1} , and 44.45 for the ADi group, respectively. Non-atopic control samples were taken from subjects who had neither a personal/family history nor any sign of atopic diseases, and the samples were selected from foreskins. An informed consent was obtained from all the patients and control individuals. The study was approved by the Medical Ethics Committee of Samsung Biomedical Research Institute.

2.2. Primary cell cultures

Punch biopsies 3–5 mm in diameter were individually obtained from the lesional skin of both the extrinsic and intrinsic types of AD patients. The specimens were briefly sterilized in 70% ethanol, minced and then treated with dispase overnight at 4 °C. For culturing the fibroblasts, the dermis was separated and placed in a solution containing 0.05% trypsin and 0.025% EDTA for 15 min at 37 °C. After vigorous vortexing, the cells were pelleted and resuspended in DMEM containing 10% FBS and 1% Antibiotic-Antimycotic (Gibco, Rockville, MD). The cells were grown at 37 °C in 5% CO₂ in an incubator. For culturing the keratinocytes, the epidermis was separated and we followed the same procedures that were applied in the fibroblasts' culture except

that we used keratinocyte media such as KGM[®] SingleQuots[®] (Cambrex, MD, USA).

2.3. Sample preparation and 2-DE

Primary cultured fibroblasts (fewer than four passages) were harvested in 50 mM Tris buffer that contained 7 M urea, 2 M thiourea, 4% CHAPS and 1× protease inhibitor cocktail (Roche). The lysates were homogenized and centrifuged at 12,000 × g for 15 min. Seven hundred units per milliliters of endonuclease (Sigma) was added to the mixture and it was stored at −70 °C after quantitation was performed by the Bradford method. For the 2D-PAGE analysis, immobilized pH gradient (IPG) strips (Amersham Biosciences) for pH 4–7 were rehydrated in swelling buffer that contained 7 M urea, 2 M thiourea, 0.4% DTT and 4% CHAPS. The lysates (500 µg) were cup-loaded onto the rehydrated IPG strips using a Multiphor II apparatus (Amersham Biosciences) for a total of 57 kVh. The two-dimensional separation was performed on 8–16% linear gradient SDS-polyacrylamide gels. Following fixation of the gels for 1 h in a solution of 40% methanol that contained 5% phosphoric acid, the gels were stained with colloidal Coomassie Blue G-250 (Sigma) for 5 h. The gels were destained in 1% acetic acid for 12 h and then they were imaged using a GS-710 imaging calibrated densitometer (Bio-Rad). The protein spot detection and 2D pattern matching were carried out using ImageMaster[™] 2D Platinum Software (Amersham Biosciences). To compare the spot densities of the normal and AD samples, more than 30 spots throughout all the gels were correspondingly landmarked and normalized. We quantified every spot density of the candidate proteins with the aid of histograms. To obtain suitable gel images, we repeated the above procedures and individually conducted two-pairs of 2D-PAGE gels for two different donors for each of the control, extrinsic and intrinsic type of AD sample.

2.4. Mass spectrometry

The protein spots excised from the gels were washed in 25 mM ammonium bicarbonate buffer (pH 7.8) that contained 50% (v/v) acetonitrile for 1 h at room temperature. The protein spots were then dehydrated in a SpeedVac for 10 min. The gel pieces were rehydrated in 10 µL (10 ng/µL) of sequencing grade trypsin solution (Promega, WI, US) that was mixed in 25 mM ammonium bicarbonate buffer at pH 7.8 and 37 °C overnight. The resulting peptides, after removing the trypsin solution, were extracted by sonication with 5 µL of 0.5% trifluoroacetic acid (TFA) that contained 50% acetonitrile. The peptide extraction was done at room temperature for 40 min. The tryptic digested peptide samples (1 µL) were mixed with equal volumes of α-cyano-4-hydroxycinnamic acid (5 mg/mL in 50% (v/v) acetonitrile/0.1% TFA), and then they were loaded onto a target plate. The MALDI-TOF mass spectra were obtained on a Voyager-DE STR mass spectrometer (PerSeptive Biosystems, MA). The peptide matching and protein searches against the SWISS-PROT and NCBI databases were performed using the ProFound program (<http://prowl.rockefeller.edu/>).

2.5. RNA extraction and cDNA synthesis

The total RNAs were isolated from the atopic and normal fibroblasts with using an easy-BLUE[™] kit (Intron, Seoul, Korea). cDNA synthesis was carried out with an M-MLV RT kit (Intron, Seoul, Korea) according to the manufacturer's instructions. Quality control for the RNA samples was carried out by performing spectrophotometry and agarose gel electrophoresis.

2.6. Real-time PCR

Real-time PCR was performed with an ABI PRISM[®] 7900 Sequence Detection System (Applied Biosys-

Table 1 Primers used in the real-time PCR

Primer	Sequence	Expected size (bp)
Caldesmon 1 isoform 5	Forward: 5'-CTGGCTTGAAGGTAGGGGTTT-3'	82
	Reverse: 5'-TTGGGAGCAGGTGACTTGTTC-3'	
Nucleophosmin 1	Forward: 5'-GGAGGTGGTAGCAAGGTTCC-3'	143
	Reverse: 5'-TTCAGTGGCGCTTTTCTTCA-3'	
Esterase D	Forward: 5'-TGCTTTGGGGGATTGCAGAAA-3'	129
	Reverse: 5'-ACCTGAGAGCCAATACAGTGC-3'	
Protein kinase C inhibitor 1	Forward: 5'-GGTGGCGACACGATCTTTG-3'	104
	Reverse: 5'-GCTTGAGGGGAAATGTCATGG-3'	
Chloride intracellular channel 4	Forward: 5'-TGAAAGCATAGGAACTGCCC-3'	177
	Reverse: 5'-ACATCCGTTTTGACTTCACTGT-3'	

tems, CA, USA). The reaction mixture for each gene contained 12.5 μ L of SYBR Green Master Mix (Applied Biosystems), 0.5 μ L cDNA, 0.5 μ L of 10 pmol of each primer in a final volume of 25 μ L. GAPDH was used at the same composition for the control quantification. The PCR amplification protocol was as follows: 50 °C for 2 min and 95 °C for 10 min, and this was followed by 40 cycles at 95 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min. In the last step, a temperature gradient between 95 and 60 °C was created for the analysis of the dissociation curves. The primers for GAPDH were 5'-TGCACCACCACTGCTTAGC-3' for the forward primer and 5'-GGCATGGACTGTGGTCATGAG-3' for the reverse primer. Each 96-well plate contained serial dilutions of cDNA (0.25, 0.5 and 1 ng), and these were used to generate the relative standard curves for the target genes. Each sample, including the control, was designed in triplicate. The primers for the target genes (Table 1) were accompanied by verification of a single gel band of the expected size and a dissociation curve analysis. For the sequential real-time PCR studies, we newly recruited four ADe patients, four ADi patients and five normal donors.

3. Results

3.1. The 2D-PAGE and mass spectrometry results of primary cultured fibroblasts from the AD patients and normal subjects

From our recent observations, we found that even under the same experimental conditions, the different lot numbers of commercial IPG strips can cause different resolutions of the 2D gels. We performed the same experiments as we did in our previous study [6] with using different lot numbers of strips. As a result, we evaluated the strip variations that showed different resolutions of the overall proteome (Fig. 1). From these images, we successfully detected several new AD-associated candidate proteins and they are listed in Table 2, even though the overall gel images were quite (Fig. 1B) or slightly different (Fig. 1C) from the previous report (Fig. 1A). The detected spots were identified and quantified by performing MALDI-TOF and spot density image analyses, respectively.

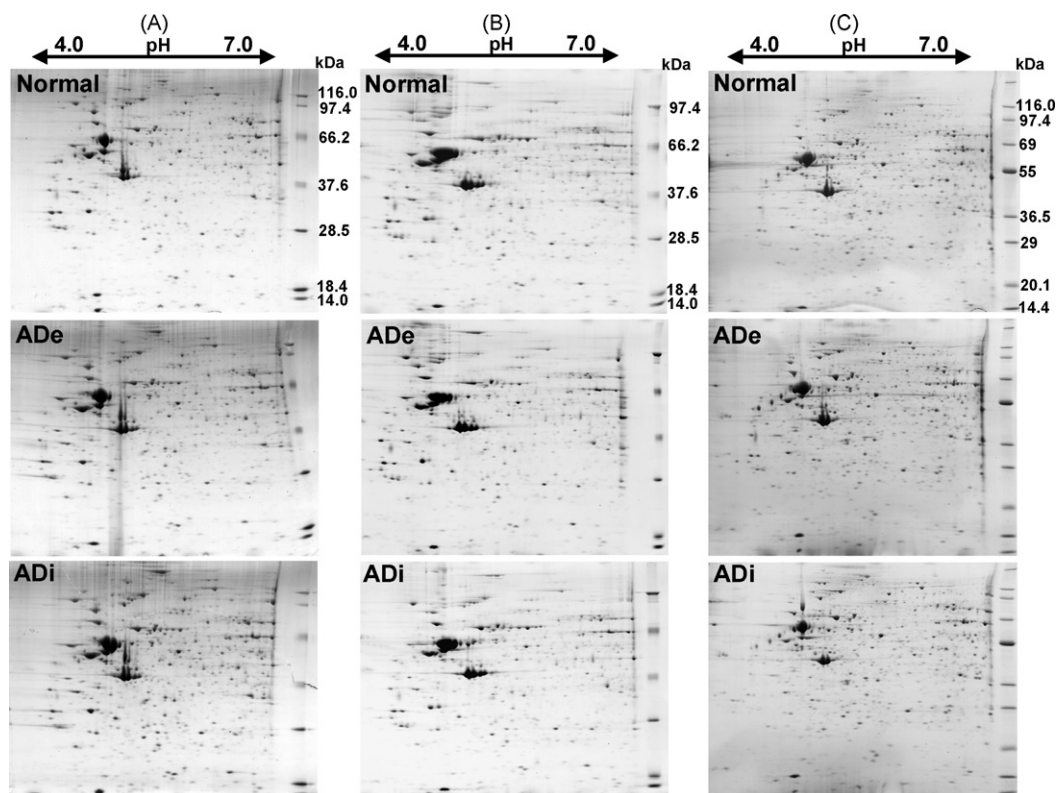


Fig. 1 2D-PAGE maps of the primary cultured fibroblasts in the pH range of 4–7 from the tissues of the normal, extrinsic type of AD and the intrinsic type of AD. The gels were stained with Coomassie Blue G-250. The labels normal, ADe and ADi indicate normal fibroblasts and fibroblasts from the extrinsic and intrinsic type of AD patients, respectively. The IPG strips with three different lot numbers are used in lanes (A)–(C). The (A) lane image was from our previous report [6].

Table 2 Proteins that were differentially altered in the atopic fibroblasts compared to the normal fibroblasts

Protein	Accession no./ database	Measured Mr (kDa)/pI	Seq. coverage (%)	No. of matched peptides	Relative amount (%) ^a
Cytovillin 2	AAA61278.1/NCBI	79/6.2	22	12	–77% ADe; –71% ADi
Caldesmon 1 isoform 5	NP_149131.1/NCBI	79/6.3	37	16	–16% ADe; –71% ADi
Nucleophosmin 1 (NPM1)	AAH09623.1/NCBI	36/4.6	33	11	–85% ADe; –75% ADi
CGI-150 protein	AAH15848.1/NCBI	31/5.4	40	13	–36% ADe; –81% ADi
Esterase D	P10768/NCBI	32/6.7	64	12	–12% ADe; –98% ADi
Protein kinase C inhibitor 1	NP_006752.1/NCBI	30/4.5	33	8	–98% ADi (–1% ADe)
Chloride intracellular channel 4 ^b	NP_039234.1/NCBI	30/5.5	55	13	–26% ADe; –99% ADi
		31/5.5	68	13	–67% ADe; –89% ADi
Glutaminase isoform C	600267/NCBI	59/6.4	32	13	–48% ADe; –76% ADi
Ubiquitin-specific protease 14	NP_005142.1/NCBI	63/5.3	45	20	315% ADi (5% ADe)

^a Data were calculated as the relative density amount (%) = (normalized AD target spot density)/(normalized control normal spot density) (%) – 100 (%).

^b Detected as multiple-spots.

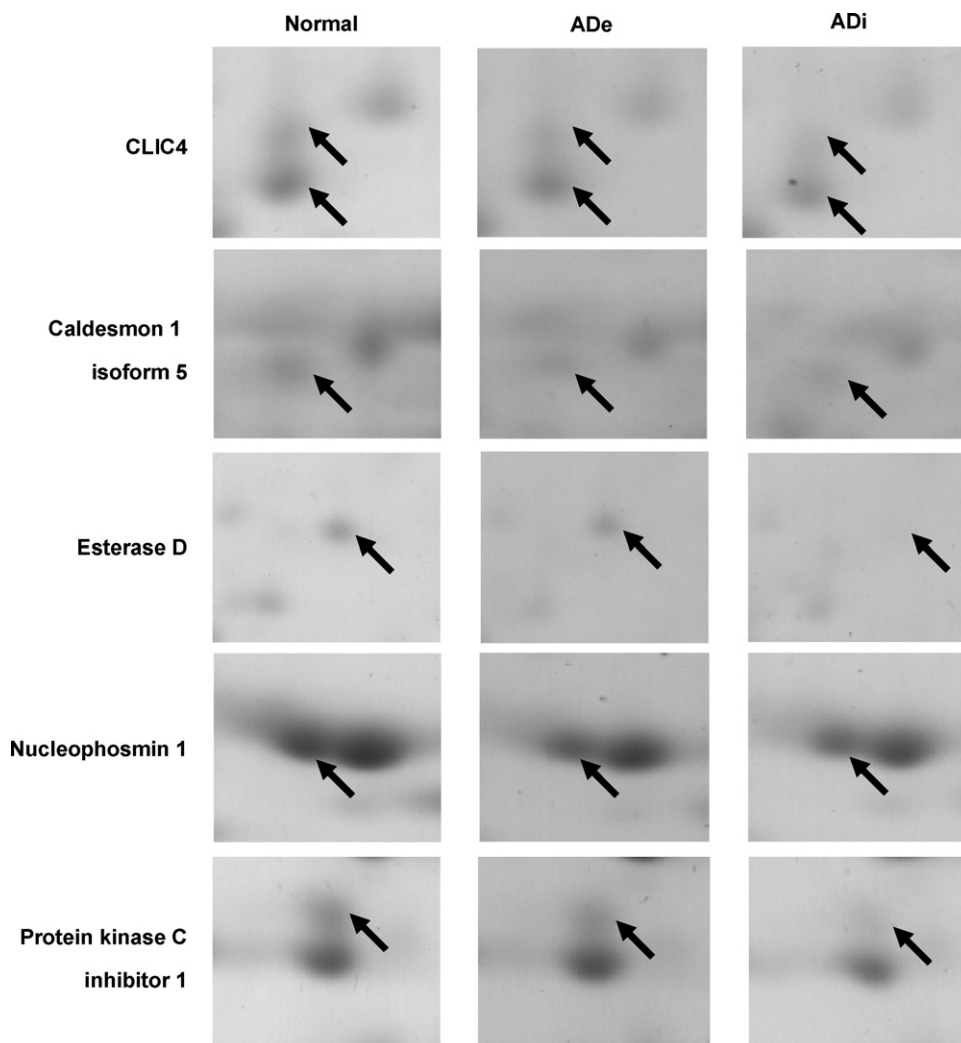


Fig. 2 2D-PAGE detections of the candidate proteins in the normal, ADe and ADi fibroblasts. Arrows indicate the target proteins, which were further identified by MALDI-TOF and database searches.

3.2. The multiple-spots detected CLIC4 and real-time PCR matching for the newly detected candidate proteins

Chloride intracellular channel 4 (CLIC4) was detected as multiple-spots that had a variety of molecular weight at the same pI value (Fig. 2). The spot density analyses showed that both spots were down-regulated in the two types of AD samples (Table 2). The real-time PCR result for CLIC4 consistently confirmed the overall down-regulated transcriptional levels in the AD-fibroblasts (Fig. 3). In our previous reports

[6,7], CLIC1 (NCC27) was detected in the AD-fibroblasts and AD-keratinocytes as a candidate protein that had defects in its post-translational modifications. In this study, we also detected a member of the p64 family.

Besides CLIC4, we newly detected several candidate proteins such as caldesmon 1 isoform 5, esterase D, nucleophosmin 1, and protein kinase C inhibitor 1, which were down-regulated in either ADe or ADi samples (Fig. 2). Among the detected proteins listed in Table 2, we selected caldesmon 1 isoform 5, nucleophosmin 1, esterase D and protein

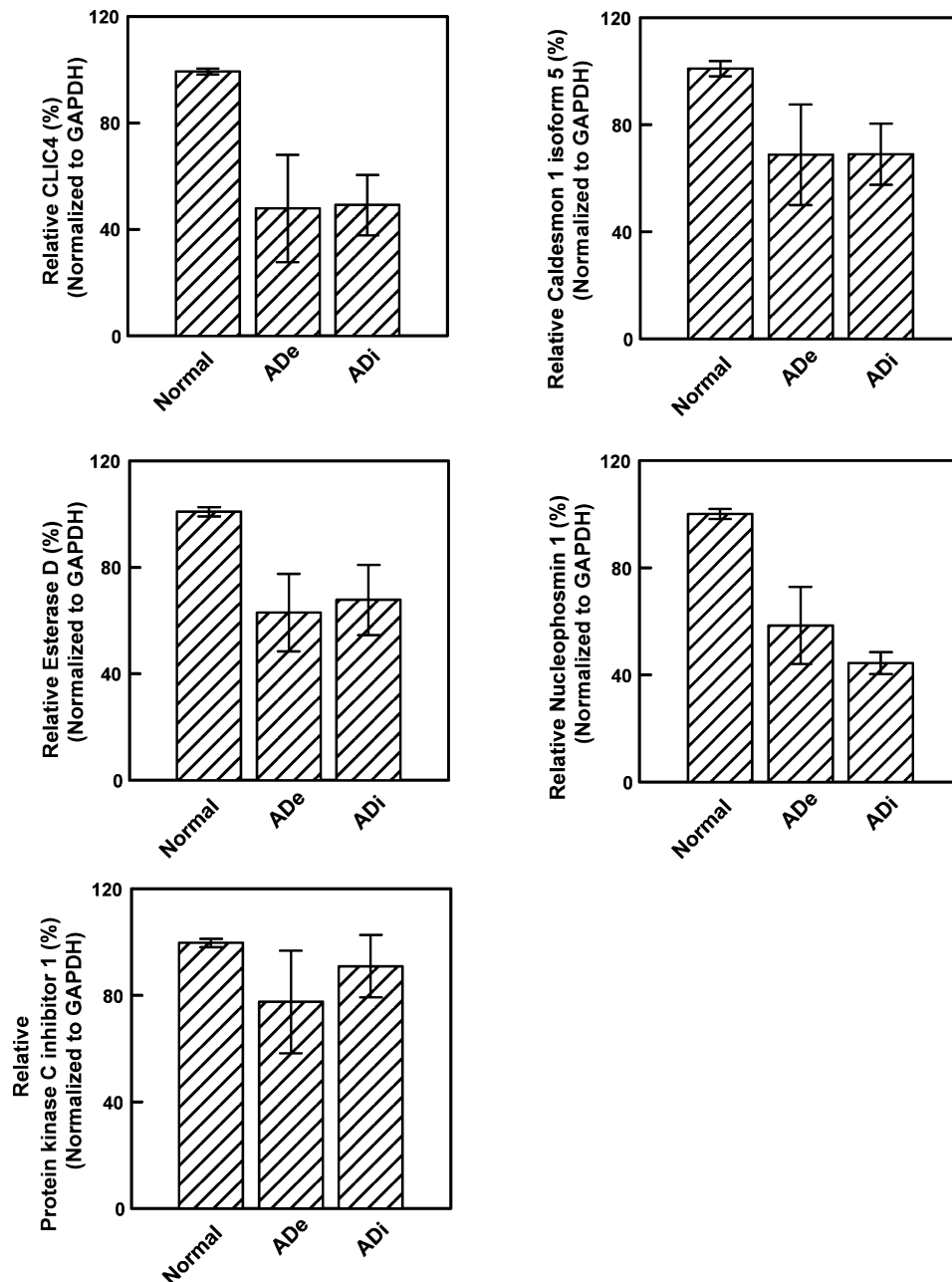


Fig. 3 Real-time PCR analyses for the AD-fibroblasts. The protein lists were selected from Table 2. PCRs were individually conducted for normal ($n = 5$), ADe ($n = 4$), and ADi ($n = 4$) fibroblasts. Data are mean values $\pm$ S.E.M.

kinase C inhibitor 1 to conduct further real-time PCRs to match them with the transcriptional levels. As a result, caldesmon 1 isoform 5, esterase D, and nucleophosmin 1 were shown to have down-regulated patterns while protein kinase C inhibitor 1 was not shown to have significant alterations in its transcriptional level (Fig. 3). It is generally recognized that some of the candidate proteins that are detected with proteomic tools are not always matched to the transcriptional levels. The matched proteins in both the 2D-PAGE and real-time PCR analyses are somehow importantly involved with AD disease.

3.3. The negative aspect of IPG strip variations in the trials of the AD-keratinocytes studies

Compared to the case of the positive aspect of the IPG strip variations that were observed during the AD-fibroblast proteomic studies, we found that IPG strip variations have negative aspects as well. In the AD-derived keratinocytes 2D-PAGE studies, we failed to get a suitable gel image for the AD patient samples due to the strip variations, and this was in

spite of getting the suitable gel image from the normal control sample (A lane in Fig. 4). Because of the suitable proteome separations of the normal sample that occurred in the initial step, we preceded with the 2D-PAGE analyses for the AD patient-derived samples under the same experimental conditions, including the usage of the same batch of commercial IPG strips. However, we failed to get suitable gel images for the AD-keratinocytes even though those strips had the same lot numbers. We examined all the individual procedures via adjusting the IEF conditions, the buffer compositions and the protein preparation procedures that were used to reproduce the gel images. However, we still could not obtain satisfactory results, and moreover, we could not reproduce the initial normal control image (B lane in Fig. 4). Only after we had changed to strips with a different lot number from the same company and conducted 2D-PAGE under the initial experimental conditions we finally achieved the proper gel images (C lane in Fig. 4). The results were reproducible and they were in agreement with further image analysis. The images shown in Fig. 4C were quite similar to our recent image data of the AD-keratinocytes [7]. These results indicate

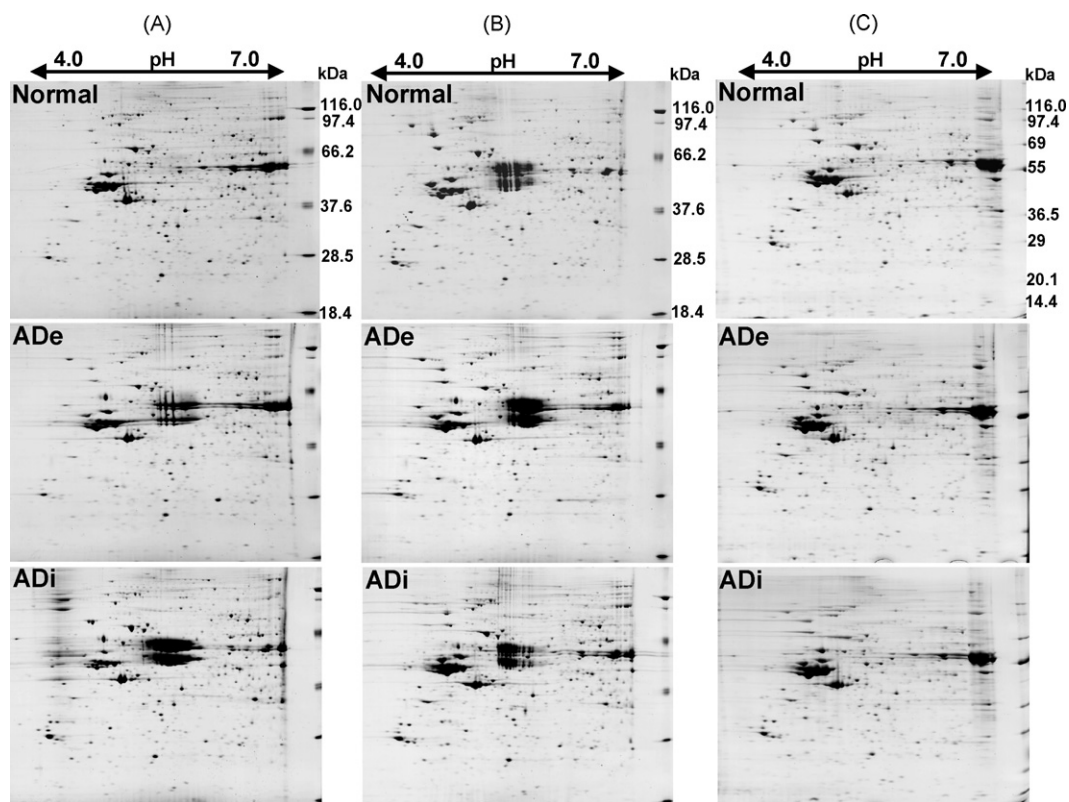


Fig. 4 2D-PAGE maps of the primary cultured keratinocytes stained with Coomassie Blue G-250 from the tissues of normal skin, the extrinsic type of AD skin and the intrinsic type of AD skin, respectively. Strips with the same lot numbers are used in (A) and (B) lanes. Strips with a different lot number, as compared to the (A) and (B) lanes, were used in (C) lane.

the negative aspects of the IPG strip problem that caused sample loss and the failure of protein separations compared to the positive results from the case of the AD-fibroblasts studies.

4. Discussion

Rapid developments of techniques for both gel-based 2D-PAGE and gel-free non-2D-PAGE have enabled researchers to glean new insights for understanding human diseases. Several factors must be considered in the case of proteomic analysis associated with diseases such as the limitations of the sample amount from the patients and the reproducibility of the experiment. In this regard, the IPG strip variations we encountered could be a factor that researcher should take into consideration. If researchers encounter the problem of reproducing gel images among their samples, we can suggest that they first consider strip variations during their trouble-shooting. In contrast, changing strips in the sequential trials with the same set of samples may increase the chances of finding a new candidate protein. Problems with commercial strip products do exist; however, these strips are still very useful for screening candidate proteins on a high-throughput basis. Although we cannot say what strips are best to use for which kinds of experiments, we can suggest that changing strips may increase the chances for finding new candidate proteins, or changing strips can be valuable for trouble-shooting the problems that happen when obtaining suitable gel images.

With the efforts of researchers, the important AD-associated factors have been gradually revealed: they can be IgE/FcεRI, antimicrobial peptides, cytokines/chemokines, proteases and their inhibitors, lipid genes, autoantigens, extracellular matrix genes, epidermal differentiation complex, etc. On the basis of these advances, new treatments have been discovered and their clinical applications for treating AD have been done. Regardless of the various approaches that are currently being used to treating AD, the number of atopic patients has greatly increased and more effective treatments that offer quick relief of severe symptoms still remain to be developed. Thus, more insights are needed into the pathogenesis of AD, in addition to elucidating the immunologic aspects of this disease. In this regard, we suggest the new AD-associated candidate proteins from our proteomic studies: cytovillin 2, caldesmon 1 isoform 5, nucleophosmin 1, CGI-150 protein, esterase D, protein kinase C inhibitor 1, chloride intracellular channel 4, and glutaminase isoform C were all detected as being down-regulated, and ubiquitin-specific protease 14

was detected as being up-regulated. From the quantification via real-time PCR at the transcriptional level, several proteins such as caldesmon 1 isoform 5, nucleophosmin 1, esterase D and chloride intracellular channel 4 were consistently matched with their translational level, and this suggests that those proteins are somehow importantly involved with AD disease. In respect of the skin's structural homeostasis, cell adhesion proteins and the extracellular matrix-associated proteins are important for external microbial infections, T cell survival and keratinocytes apoptosis [9]. Cytoovillin (ezrin) plays a key role in cell surface structure adhesion, cell migration and organization as a cytoskeletal protein [10,11]. Caldesmon 1 has the binding activities to calmodulin, actin, tropomyosin, myosin and phospholipids, and all of these are constituents of the skin structure [12,13]. Nucleophosmin 1 has been shown to be a coactivator of NF-κB during the induction of manganese superoxide dismutase and it is an early responder to DNA damage for preventing the premature activation of p53 in response to stress [14,15]. The lymphoblasts obtained from Fanconi anemia (FA) patients expressed low levels of nucleophosmin 1 [16]. In this study, we also showed the down-regulated patterns of nucleophosmin 1 in the AD patients-derived fibroblasts. Since nucleophosmin 1 is a NF-κB binding factor, it could regulate the cytokine-mediated skin inflammation that has been observed in the pathogenesis of AD. Esterase D has a serine esterase activity and it is a cytoplasmic membrane-bound vesicle component. In a couple of previous reports, linkage studies showed that esterase D is associated with some human diseases such as Wilson's disease and retinoblastoma [17,18]. Chloride intracellular channel 4 protein may play a role in regulating the cytoskeletal organization during the cell cycle and it is associated with the microtubule cytoskeleton; these functions are beyond its proposed role as a chloride channel [19]. Chloride intracellular channel 4 is translocated to the nucleus in response to cellular stress and it accelerates apoptosis, and it is dysregulated in the myofibroblasts of breast carcinoma [20,21].

In conclusion, our 2D-PAGE studies on the profile changes in the protein expression patterns of AD-fibroblasts versus the fibroblasts of normal control subjects revealed several additional important proteins that are involved in the pathogenesis of AD. We discovered these findings due to the IPG strip variations, and the correlation between the transcriptional and translational levels of certain candidate proteins suggests that they play important roles in the dermis of AD patients. We also showed the negative aspects of the strip variations in the case of the AD-keratinocytes studies.

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References

- [1] Banks RE, Dunn MJ, Hochstrasser DF, Sanchez JC, Blackstock W, Pappin DJ, et al. Proteomics: new perspectives, new biomedical opportunities. *Lancet* 2000;356:1749–56.
- [2] Fathman CG, Soares L, Chan SM, Utz PJ. An array of possibilities for the study of autoimmunity. *Nature* 2005;435: 605–11.
- [3] Abramovits W. Atopic dermatitis. *J Am Acad Dermatol* 2005;53:S86–93.
- [4] Simpson EL, Hanifin JM. Atopic dermatitis. *J Am Acad Dermatol* 2005;53:115–28.
- [5] Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New insights into atopic dermatitis. *J Clin Invest* 2004;113:651–7.
- [6] Park YD, Kim SY, Jang HS, Seo EY, Namkung JH, Park HS, et al. Towards a proteomic analysis of atopic dermatitis: a two-dimensional-polyacrylamide gel electrophoresis/mass spectrometric analysis of cultured patient-derived fibroblasts. *Proteomics* 2004;4:3446–55.
- [7] Park YD, Jang HS, Kim SY, Ko SK, Lyou YJ, Lee DY, et al. Two-dimensional electrophoretic profiling of atopic dermatitis in primary cultured keratinocytes from patients. *Proteomics* 2006;6:1362–70.
- [8] Jeong CW, Ahn KS, Rho NK, Park YD, Lee DY, Lee JH, et al. Differential in vivo cytokine mRNA expression in lesional skin of intrinsic vs. extrinsic atopic dermatitis patients using semiquantitative RT-PCR. *Clin Exp Allergy* 2003;33:1717–24.
- [9] Akdis CA, Akdis M, Trautmann A, Blaser K. Immune regulation in atopic dermatitis. *Curr Opin Immunol* 2000;12:641–6.
- [10] Martin TA, Harrison G, Mansel RE, Jiang WG. The role of the CD44/ezrin complex in cancer metastasis. *Crit Rev Oncol Hematol* 2003;46:165–86.
- [11] Srivastava J, Elliott BE, Louvard D, Arpin M. Src-dependent ezrin phosphorylation in adhesion-mediated signalling. *Mol Biol Cell* 2005;16:1481–90.
- [12] Mirzapoiazova T, Kolosova IA, Romer L, Garcia JG, Verin AD. The role of caldesmon in the regulation of endothelial cytoskeleton and migration. *J Cell Physiol* 2005;203:520–8.
- [13] Li Y, Lin JL, Reiter RS, Daniels K, Soll DR, Lin JJ. Caldesmon mutant defective in Ca(2+)-calmodulin binding interferes with assembly of stress fibers and affects cell morphology, growth and motility. *J Cell Sci* 2004;117:3593–604.
- [14] Dhar SK, Lynn BC, Daosukho C, Clair DKSt. Identification of nucleophosmin as an NF-kappaB co-activator for the induction of the human SOD2 gene. *J Biol Chem* 2004;279:20219–8209.
- [15] Maiguel DA, Jones L, Chakravarty D, Yang C, Carrier F. Nucleophosmin sets a threshold for p53 response to UV radiation. *Mol Cell Biol* 2004;24:3703–11.
- [16] Pang Q, Christianson TA, Koretsky T, Carlson H, David L, Keeble W, et al. Nucleophosmin interacts with and inhibits the catalytic function of eukaryotic initiation factor 2 kinase PKR. *J Biol Chem* 2003;278:41709–17.
- [17] Lee EY, Lee WH. Molecular cloning of the human esterase D gene, a genetic marker of retinoblastoma. *Proc Natl Acad Sci USA* 1986;83:6337–41.
- [18] Yuzbasiyan-Gurkan V, Wagnitz S, Blanton SH, Brewer GJ. Linkage studies of the esterase D and retinoblastoma genes to canine copper toxicosis: a model for Wilson disease. *Genomics* 1993;15:86–90.
- [19] Berryman MA, Goldenring JR. CLIC4 is enriched at cell–cell junctions and colocalizes with AKAP350 at the centrosome and midbody of cultured mammalian cells. *Cell Motil Cytoskeleton* 2003;56:159–72.
- [20] Suh KS, Mutoh M, Nagashima K, Fernandez-Salas E, Edwards LE, Hayes DD, et al. The organellar chloride channel protein CLIC4/mtCLIC translocates to the nucleus in response to cellular stress and accelerates apoptosis. *J Biol Chem* 2004;279:4632–41.
- [21] Ronnov-Jessen L, Villadsen R, Edwards JC, Petersen OW. Differential expression of a chloride intracellular channel gene, CLIC4, in transforming growth factor-beta1-mediated conversion of fibroblasts to myofibroblasts. *Am J Pathol* 2002;161:471–80.

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