ORIGINAL

Identification of ultraviolet B-sensitive genes in human peripheral blood cells

Eun Ju Jung*, Tomoko Kawai†, Hwan Ki Park§, Yoshiaki Kubo*, Kazuhito Rokutan†, and Seiji Arase*

*Departments of Dermatology and †Department of Stress Science, Institute of Health Biosciences, the University of Tokushima Graduate School ; and §Division of Enzyme Pathophysiology, The Institute for Enzyme Research, the University of Tokushima, Tokushima, Japan

Abstract : Ultraviolet B (UVB) is a serious irritant for the skin and increases a risk for skin cancer. To identify UVB-sensitive genes in peripheral blood, 11 healthy male volunteers were exposed to 0.3 J/cm^2 of narrow-band (NB)-UVB, about half of minimal erythema dose (MED) in Japanese, and gene expression in blood was analyzed at 4 h, 24 h, 4 d and 7 d after the irradiation using microarray carrying oligonucleotide probes for 2,000 stress-responsive genes. RNA prepared before the irradiation was used as a reference control. Microarray analysis identified 21 genes as UVB-responsive genes with a peak at 24 h in 6 subjects, and real-time PCR validated the significant down-regulation of 9 (*ABCB10*, *ATF1*, *ABCD3*, *TANK*, *FAS*, *SLC30A9*, *CHUK*, *CASP1*, and *ABCE1*) out of the 21 genes in 11 subjects. Considering sensitive and characteristic features of 9 marker genes, they may be useful indicators for monitoring systemic response to UVB irradiation.
J. Med. Invest. 55 : 204-210, August, 2008

Keywords **:** *ultraviolet B irradiation, skin damage, peripheral blood cells, gene expression, marker genes*

INTRODUCTION

The skin is a physiological barrier against environmental stressors such as sunlight, pathogens, and chemical/physical insults. One of the predominant environmental stressors for the skin is ultraviolet (UV) radiation, especially ultraviolet B (UVB) with a wavelength range between 290 and 320 nm (1). To protect the skin against the DNA-damaging effects of UVB, the skin is endowed with highly complicated cellular programs, including cell-cycle arrest, DNA repair, and apoptosis (2, 3). To provide systemic protection, skin cells also have numerous defense mechanisms, presumably evolving from a network of intracellular communications coordinated by messages from the local neuro-endocrine-immune system (4).

Exposure to UV radiation suppresses systemic immune responses largely by modulating cytokine gene expression in skin keratinocytes (5-8). However, information on the molecular mechanism of the UV-induced response has been provided mostly through the use of cultured cell lines (9-11), and those studies *in vitro* may not evenly reflect local and systemic responses *in vivo* (12).

The local skin defense machinery communicates with circulating blood leukocytes by producing cytokines and other inflammatory mediators (13). Peripheral blood leukocytes express receptors for these mediators and also produce various biologically active molecules. Thus, peripheral blood leukocytes may be one of appropriate targets for the

Received for publication March 14, 2008 ; accepted May 1, 2008.

Address correspondence and reprint requests to Seiji Arase, MD, PhD., Department of Dermatology, Institute of Health Biosciences, the University of Tokushima Graduate School, Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +81-88-632-0434.

detection and assessment of local or systemic responses to UVB irradiation.

Acute UVB irradiation causes not only sunburn but also fatigue, and decreases local and systemic immunities. Chronic and persistent exposure to UVB is carcinogenic. To prevent these hazardous effects, it is potentially useful to identify markers sensitive to UVB irradiation in peripheral blood. In this study, we recruited healthy male volunteers, exposed them to a sub-clinical dose of UVB, and examined gene expression profiles in peripheral blood cells (PBC) using a microarray carrying oligonucleotide (80 mer) probes for 2,000 stress-responsive genes. Here we show 9 genes identified as sensitive markers for UVB stress.

MATERIALS AND METHODS

Subjects

Eleven healthy male Japanese volunteers with either skin type III or IV were recruited for the study. They were all in good physical health, nonsmokers, and receiving no medications (median age 27.2 years, range 23-39 years). The study was approved by the Human Study Committee of Tokushima University Hospital. A written informed consent was obtained from each participant prior to the study.

Narrow-band (NB) UVB irradiation and blood collection

Eleven subjects were exposed to NB-UVB of $0.3 J/cm^2$, about half of minimal erythema dose (MED : the smallest amount of energy to produce a perceptible erythema) in Japanese, on the entire body except the face and genital region in a special cabinet equipped with 20 NB-UVB bulbs emitting a very short range of wavelengths from 311 to 315 nm exclusively (UV7001K-TL01, Waldmann, Villingen-Schwenningen, Germany). The irradiation was performed between 13 : 00 to 14 : 00. Peripheral venous blood (12 ml) was taken from each subject immediately before and 4 h, 24 h, 4 d, and 7 d after UVB irradiation. Two ml of blood was used for total and differential leukocyte counts, and remaining 10 ml of the blood was poured into PAXgene™ Blood RNA tubes (Becton Dickinson, Franklin Lakes, NJ).

Construction of microarray

We had originally developed a complementary DNA (cDNA) microarray specifically designed to assess stress response in peripheral blood cells (14, 15). This carried cDNA probes for 1,467 genes that were detected in total RNA from peripheral blood by reverse transcriptase (RT)-PCR. In this study, we constructed a new microarray carrying 80-mer oligonucleotide probes for the above 1,467 genes and newly added 400 genes that are categorized into drug metabolism (see http://www.hitachi.co.jp/LS/ for the full list of genes). These oligonucleotide probes were designed using original software from Hitachi, Ltd. (Saitama, Japan). The microarray showed high reproducibility with a mean coefficient of variation of less than 10%, and the dynamic ranges were three orders of magnitude. This microarray was purchased from Hitachi, Ltd.

RNA preparation, amplification, and hybridization

RNA was isolated from PAXgene™ Blood RNA tubes using a PAXgene blood RNA kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Contaminated DNA was removed using a DNase-kit (Qiagen). The quality of resultant RNA was checked with an Agilent 2100 Bioanalyzer using an RNA 6000 Nano Labchip kit (Agilent Technologies, Palo Alto, CA). Five micrograms of total RNA from randomly selected 6 subjects were reverse transcribed with an oligo dT primer conjugating T7 sequence. Yielded first strand cDNA complementary to poly (A) RNA was amplified by using MEGAscript T7 *in vitro* RNA transcription kit (Applied Biosystems, Foster City, CA). Amplified RNA (6 μg) was reverse transcribed by using random hexamers and aminoallyl-dUTP. The synthesized cDNA was labeled with dye (NHS-ester Cy5 or Cy3 ; Amersham Biosciences, Piscataway, NJ). Cy5-cDNAs from each blood sample collected at the indicated times after UV irradiation were mixed with the equivalent amount of Cy3-cDNAs, which was from each subject prior to UV irradiation. Hybridization of the oligonucleotide microarray was performed at 62°C for 12 h. After washing, fluorescence intensity at each spot was assayed using a scanner (ScanArray 5000 ; GSI-Lumonics, Billerica, MA).

Data analysis

Signal intensities of Cy5 and Cy3 were quantified and analyzed by subtracting backgrounds using QuantArray software (GSI-Lumonics, Billerica, MA). The intensity values for duplicate oligoDNA probes were averaged. Following global normalization, we selected 1,095 genes having fluorescence

intensities higher than the cut-off value of 300 for either Cy5 or Cy3 conditions among all 24-paired samples. In order to consider the quality criteria of our microarray chip, we performed comparative experiments by hybridizing the same 2 samples on one microarray chip after labeling Cy5 and Cy3 dye separately (self-self test). Based on results of these experiments, we decided that cut-off values higher than 300 are sufficient for analysis. The relative expression values (Cy5/Cy3) for 1,095 genes were subjected to hierarchical clustering using GeneSpring 7.3 software (Agilent), and similarity was measured by standard correlation. After Cy5/Cy3 ratios of 1,095 genes were transformed to logarithms, statistical significance between UV irradiation and non-irradiated controls was examined by the paired *t*-test using Cyber-T stats program written in the R stats language (available at http : // visitor.ics.uci.edu/genex/cybert/help/index.html). Statistical significance was defined as a Bonferroni-corrected P value of < 0.05.

Quantitative real-time RT-PCR

Total RNA (0.5 μg) was prepared as a template for cDNA synthesis using the SuperScript™ III first-strand synthesis system for RT-PCR (Invitrogen, Carlsbad, CA). The mRNA levels of target genes based on GenBank association numbers (*CDC34*, TaqMan assay ID Hs00362082_m1 ; *GSTP1*, Hs 00168310_m1 ; *ABCB10*, Hs00429240_m1 ; *SLC4A7*, Hs00186192_m1 ; *ATF1*, Hs00270896_m1 ; *ABCD3*, Hs00161065_m1 ; *CAPN7*, Hs00204734_m1 ; GABPB2, Hs00242573_m1 ; HSPH1, Hs00971475_ m1 ; GABPA, Hs01022023_m1 ; CCNC, Hs 00193177_m1 ; TANK, Hs00370305_m1 ; FAS, Hs 00531110_m1 ; SLC35A5, Hs00215733_m1 ; ATP6 V1C1, Hs00184625_m1 ; POLR2B, Hs00265358_ m1 ; SLC30A9, Hs00197118_m1 ; CHUK, Hs 00175141_m1 ; CASP1, Hs00169146_m1 ; ABCE1, Hs01003010_g1 ; HAT1, Hs00186320_m1 ; GAPDH, Hs99999905_m1) were analyzed by quantitative real-time PCR using TaqMan gene expression assays and ABI-PRISM 7500 sequence detection system (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous quantity control. Data were analyzed using SDS 2.3 software (Applied Biosystems). The threshold cycle (Ct) for each gene was calculated by the standard curve method. Quantity values were finally normalized to GAPDH mRNA expression. After the relative ratio of each mRNA expression level between pre- and post-irradiation was calculated, the paired *t*-test was performed to compare the mean of relative ratios for each gene.

RESULTS

Changes in gene expression in peripheral blood cells after UVB irradiation

A single exposure to 0.3J/cm^2 NB-UVB did not cause any change on the skin in any of the subjects. To detect UVB-sensitive genes, among the 11 volunteers exposed to the irradiation, we randomly selected 6 subjects and measured time-dependent changes in their gene expression profiles using RNAs prepared prior to the irradiation as individual controls. As shown in the hierarchical clustering map of the 1,095 genes (Fig. 1), 2 subjects (Nos. 2 and 3) had a similar expression change. This change was also detected at one time point (24 h) in subject 4. However, response of gene expression differed among the other subjects (Nos. 1, 5, and 6), suggesting a significant individual variability of sensitivity and gene expression response to NB-UVB irradiation. In all of the cases, most profound changes in gene expression were observed 24 h after the irradiation. In spite of the variations, we could identify 21 genes whose mRNAs were significantly up- or down-regulated in all 6 subjects at 24 h after the irradiation (Table 1). None of the genes were sig-

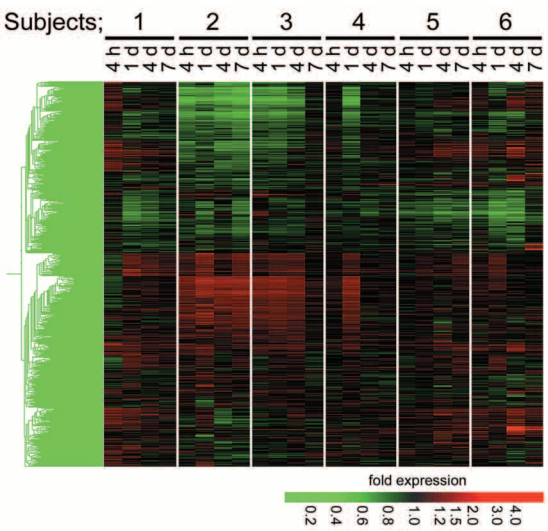

Fig. 1. Hierarchical clustering of gene expression pattern in peripheral blood cells after irradiation by 0.3 J/cm^2 NB-UVB. Whole blood RNA collected at the indicated times after irradiation by 0.3 J/cm^2 NB-UVB (Cy5) were applied to oligonucleotide microarray together with reference RNA collected immediately before irradiation (Cy3) in each 6 volunteer. Relative expression values (Cy5/Cy3) were subjected to a hierarchical clustering and shown in intensity of red and green.

Table 1. List of putative NB-UVB-responsive genes.

Gene symbol	Gene name	GenBank accession number	Fold change (mean ± SD)	*P* value
CDC34	cell division cycle 34	L22005.1	1.99 ± 0.45	1.73E-05
GSTP1	glutathione S-transferase pi	X06547.1	1.96 ± 0.43	1.20E-05
ABCB10	ATP-binding cassette, sub-family B, member 10	U18237.1	0.59 ± 0.10	3.23E-05
SLC4A7	solute carrier family 4, member 7	NM_003615.2	0.52 ± 0.12	4.59E-06
ATF1	activating transcription factor 1	NM_005171.1	0.54 ± 0.12	6.30E-06
ABCD3	ATP-binding cassette, sub-family D, member 3	M81182.1	0.42 ± 0.13	1.93E-06
CAPN7	calpain 7	NM_014296.1	0.54 ± 0.13	3.78E-05
GABPB2	GA binding protein transcription factor, beta subunit 2	NM_005254.2	0.53 ± 0.14	2.51E-05
HSPH1	heat shock 105kDa/110kDa protein 1	AB003333.1	0.49 ± 0.18	2.94E-05
GABPA	GA binding protein transcription factor, alpha subunit	NM_002040.1	0.52 ± 0.13	3.77E-05
CCNC	cyclin C	M74091.1	0.43 ± 0.19	8.77E-06
TANK	TRAF family member-associated NF-κB activator	U63830.1	0.53 ± 0.13	2.94E-05
FAS	Fas (TNF receptor superfamily, member 6)	X63717.1	0.44 ± 0.12	3.28E-06
SLC35A5	solute carrier family 35, member A5	NM_017945.2	0.50 ± 0.18	1.34E-05
ATP6V1C1	ATPase, H^+ transporting, lysosomal, V1 subunit C1	J05682.1	0.40 ± 0.19	4.34E-06
POLR2B	polymerase (RNA) II polypeptide B	X63563.1	0.45 ± 0.20	2.54E-05
SLC30A9	solute carrier family 30, member 9	NM_006345.2	0.47 ± 0.19	1.47E-05
CHUK	conserved helix-loop-helix ubiquitous kinase, IKK1	AF009225.1	0.53 ± 0.09	7.99E-06
CASP1	caspase 1, apoptosis-related cysteine peptidase	U13699.1	0.58 ± 0.08	4.58E-05
ABCE1	ATP-binding cassette, sub-family E, member 1	X74987.1	0.55 ± 0.13	3.62E-05
HAT1	histone acetyltransferase 1	NM_003642.1	0.44 ± 0.20	3.47E-05

nificantly regulated in all 6 subjects at 4 h or 7 d after the irradiation. Only one gene (*HSPH1*) included in the 21 genes significantly changed its expression level at 4 d after the irradiation. The identified 21 genes were likely to be sensitive universal marker genes, and most were detected as down-regulated genes to the lowest levels at 24 h (Fig. 2, upper panel).

Even at about half MED, NB-UVB irradiation may leads to a shift in the subpopulation of leukocytes ; therefore, the changes found in mRNA levels of whole blood might reflect a shift between leukocyte subpopulations. However, we could not detect any common, significant changes in total and differential leukocyte counts (data not shown). We had already purified neutrophil-, monocyte-, T cell-, and B cell-enriched fractions and identified genes preferentially expressed in each fraction using a whole human genome microarray carrying 44,000 oligonucleotide probes (Agilent) (see supplementary Table S1 in ref. (13)). The microarray used in this study carries 3 neutrophil markers, 4 monocyte markers, 8 B cell markers, and 9 T cell markers. We tested how mRNA levels of these 24 cell fraction-specific genes were modified after the UVB irradiation. T cell fraction-specific genes were seemed to be generally down-regulated (Fig. 2 lower panel), while none of them was included in the commonly and significantly changed genes.

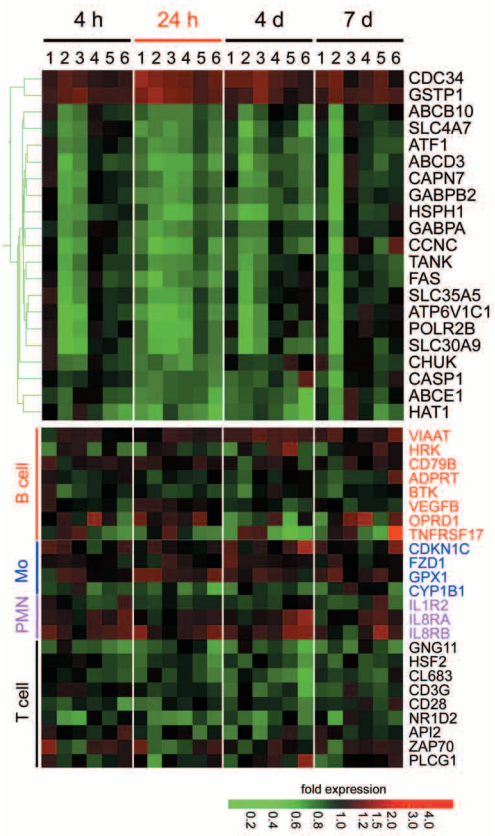

Fig. 2. Identification of 21 marker genes after irradiation by 0.3 J/cm^2 NB-UVB. The paired *t*-test (Bonferroni corrected *p* value < 0.05) revealed that 21 genes were commonly and significantly changed between before and 24 h after irradiation by 0.3 J/cm^2 NB-UVB in 1,095 genes. Time-dependent changes in relative expression levels of 21 genes are shown in the upper panel. Each leukocyte marker genes are referred in the lower panel (Mo ; monocyte, PMN ; neutrophil). Gene symbols are listed on the right.

Validation of microarray data by quantitative real-time PCR

Based on microarray findings, we performed quantitative real-time PCR to validate the putative UVB-sensitive 21 genes. In this case, mRNA levels were compared between those immediately before (control) and 24 h after NB-UBV irradiation (0.3 J/cm^2) in all 11 subjects. Real-time PCR reproduced the findings by microarray of a significant reduction in the mRNA level of 9 genes (*ABCB10*, *ATF1*, *ABCD3*, *TANK*, *FAS*, *SLC30A9*, *CHUK*, *CASP1*, and *ABCE1*) in response to a half erythemogenic dose of UVB, while we could not confirm any significant changes in the other 12 mRNA levels by real-time PCR (Fig. 3). Among the down-regulated 9 genes, 4 genes encode essential regulators of nuclear factor (NF)-κB and apoptosis ; these are a conserved helix-loop-helix ubiquitous kinase (*CHUK*), TNF receptor-associated factor (TRAF) family member-associated nuclear factor (NF)-κB activator (*TANK*), Fas (*FAS*), and caspase 1 (*CASP1*).

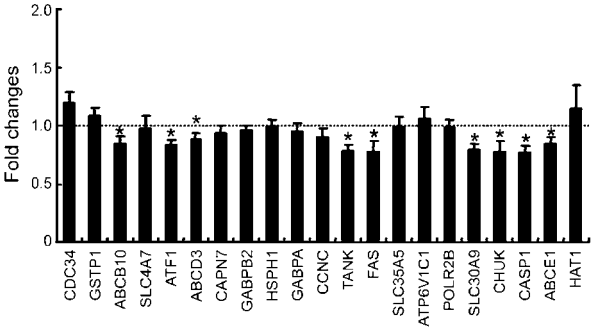

Fig. 3. Validation of UVB-responsive 21 genes. The mRNA levels of putative UVB-responsive 21 genes listed in Table 1 were measured in sample of 24 h after irradiation of NB-UVB by quantitative real-time PCR. GAPDH mRNA was used as an endogenous quantity control. The fold changes in relative ratios (each mRNA level/GAPDH mRNA) are shown. Values are means ± S. E. M. (n = 11).
*Significantly different compared with that before the irradiation ($P < 0.05$ by the paired t-test)

DISCUSSION

In addition to wavelength, UV-induced injury depends on many variables including dose, skin types, and characteristics of the skin tissue (16). Individual sensitivity, probably due to genetic factors, is also an important determinant of the susceptibility to UV irradiation.

In this study, after a single exposure to a half MED of NB-UVB (0.3 J/cm^2), time-dependent changes in gene expression of peripheral blood cells were precisely examined up to 7 d in 6 subjects using RNA prepared from each subject immediately before the irradiation as an individual reference control. The routine of daily lives of our subjects was similar, and they had no regular outdoor activities. Even these relatively homogenous subjects displayed inter-individual variations following exposure to the same UVB dose. Among the heterogeneous responses, we identified 21 genes as commonly UVB-sensitive genes. Although there was inconsistency between microarray and real-time PCR analyses, 9 genes were confirmed to be significantly down-regulated in response to the irradiation of 0.3 J/cm^2 NB-UVB by both analyses.

At present, two different technologies are commonly used for preparation of RNA from whole blood and its leukocyte compartments (17). RNA from whole blood cells contains a large amount of hemoglobin mRNAs that disturb specific and sensitive hybridization reactions (18). However, the *in vitro* handling required for isolation of distinct leukocyte compartments stimulates *ex vivo* expression and degradation of mRNAs for a number of genes, particularly stress-responsive genes (14, 19-20). Therefore, to detect UVB-sensitive genes, we used the whole blood RNA preparation method.

UV light activates distinct signaling pathways and transcription factors, leading to changes in gene expression in the skin (12, 17, 21-24). Upon UVB stress, keratinocytes produce numerous cytokines and inflammatory mediators, including production of interleukin (IL)-1β, tumor necrosis factor (TNF)-α and reactive oxygen species (25). These mediators subsequently activate NF-κB, JunD/c-Fos and p53 (26). In turn, activation of NF-κB has been reported to result in transactivation of a number of target genes, including those for IL-1, IL-6, IL-8, GRO-1, TNF-α and COX-2 (27). UVB also suppresses trafficking of lymphocytes and functions of T and natural killer cells (28). Since UV does not effectively penetrate into the dermis where blood vessels are located, inflammatory mediators produced by skin cells are believed to act on PBC and evoke systemic response (5-8). Among the 9 genes confirmed by microarray and real-time PCR to be significantly down-regulated, *ATF1* encodes activating transcription factor 1, and this transcription factor together with the cAMP response element binding protein regulates the stress-induced transcription of several immediate early genes (29). It should be noted that among the confirmed 9 genes,

4 genes (*CHUK*, *TUNK*, *CASP1*, and *FAS*) encode molecules essential for activation of NF-κB or apoptosis. *CHUK* encodes an inhibitor of NF-κB kinase α(IKK-α). IKK-α, a component of a cytokine-activated protein complex that is an inhibitor of the NF-κB complex, phosphorylates IκB leading to the degradation of the inhibitor via the ubiquination pathway, thereby activating NF-κB. *TUNK* encodes a RAF family member-associated NF-κB activator (TRAF2 ; TNF receptor-associated factor 2). TRAF2 associates with and transduces signals from members of the TNF receptor superfamily. TRAF2 binds to the TNF receptor death domain (TRADD) and recruits NF-κB-inducing kinase (NIK) leading to phosphorylation of IκB. *CASP1* encodes caspase 1, apoptosis-related cysteine peptidase (IL-1β convertase). Fas is a member of the TNF-receptor superfamily containing the death domain and plays a central role in the regulation of apoptosis. This receptor also activates NF-κB, MAPK3/ERK1, and MAPK8/JNK. The 9 genes also included *SLC30A9*, encoding a recently identified nuclear receptor coactivator interacting with β-catenin (30) and 3 genes (*ABCB10*, *ABCD3*, and *ABCE1*) encoding members of the superfamily of ATP-binding cassette (ABC) transporters.

Because the 4 identified genes (*CHUK*, *TUNK*, *CASP1*, and *FAS*) were closely related to activation of NF-κB and apoptosis, we additionally performed real-time PCR measurement of mRNA levels of other NF-κB activation associated genes such as Toll-like receptor (TLR) family members (*TLR1*, *TLR2*, *TLR4*, and *TLR5*) and 2 apoptosis-regulating factors (*BCL2A1* and *BCL2L11*) all of which are expressed in peripheral leukocytes. We also measured changes in mRNA levels of HSP60 and stress-inducible HSP70 to assess stress response. However, none of these 8 genes changed their mRNA levels after exposure to 0.3 J/cm^2 of NB-UVB (data not shown), suggesting the high sensitivity of 9 marker genes to UVB irradiation.

Dermatologists recommend a variety ways to limit sun exposure to avoid UV-induced injury. Despite the extensive use of sunscreens, the incidence of skin cancer has been increasing. The efficacy of sunscreens is generally assessed using the sun protection factor (SPF) as an indicator. Evaluation of SPF is based solely on the ability to prevent UV-induced perceptible erythema. However, our results suggest that even about half MED of UVB may evoke significant changes in the expression of distinct genes in PBC. Reliable indicators should evaluate not only the protective effect against erythema, but also potency of protection against UV-induced systemic immunological and mutational effects. In this context, sensitive and characteristic features of 9 marker genes may be useful indicators for monitoring systemic response to UVB irradiation.

REFERENCES

1. Hahn WC, Weinberg RA : Modelling the molecular circuitry of cancer. Nat Rev Cancer 2 : 331-341, 2000
2. Chow J, Tron VA : Molecular aspects of ultraviolet radiation-induced apoptosis in the skin. J Cutan Med Surg 9 : 289-295, 2005
3. Yaar M, Gilchrest BA : Photoageing : mechanism, prevention and therapy. Br J Dermatol 157 : 874-887, 2007
4. Slominski A, Wortsman J : Neuroendocrinology of the skin. Endocr Rev 21 : 457-487, 2000
5. Rivas JM, Ullrich SE : Systemic suppression of delayed-type hypersensitivity by supernatants from UV-irradiated keratinocytes : An essential role for keratinocyte-derived IL-10. J Immunol 149 : 3865-3871, 1992
6. Rivas JM, Ullrich SE : The role of IL-4, IL-10, and TNF-α in the immune suppression induced by ultraviolet radiation. J Leukoc Biol 56 : 769-775, 1994
7. Schwarz T, Urbanski A, Luger TA : Ultraviolet light and epidermal cell derived cytokines. In : T.A. Luger, T. Schwarz (Eds), Epidermal growth factors and cytokines, Marcel Dekker Inc, New York, 1994, pp.303-363
8. Kondo S, Kooshesh F, Sauder DN : Penetration of keratinocyte-derived cytokines into basement membrane. J Cell Physiol 171 : 190-195, 1997
9. Ullrich SE, Schmitt DA : Exposure to ultraviolet radiation causes dendritic cells/macrophages to secrete immune-suppressive IL-12p40 homodimers. J Immunol 165 : 3162-3167, 2000
10. Komura K, Hasegawa M, Hamaguchi Y, Saito E, Kaburagi Y, Yanaba K, Kawara S, Takehara K, Seki M, Steeber DA, Tedder TF, Sato S : Ultraviolet light exposure suppresses contact hypersensitivity by abrogating endothelial intercellular adhesion molecule-1 up-regulation at the elicitation site. J Immunol 171 : 2885-2862, 2003
11. Li-Weber M, Treiber MK, Giaisi M, Palfi K,

Stephan N, Parg S, Krammer PH : Ultraviolet irradiation suppresses T cell activation via blocking TCR-mediated ERK and NF-κB signaling pathways. J Immunol 175 : 2132-2143, 2005
12. Enk CD, Jacob-Hirsch J, Gal H, Verbovetski I, Amariglio N, Mevorach D, Ingber A, Givol D, Rechavi G, Hochberg M : The UVB-induced gene expression profile of human epidermis *in vivo* is different from that of cultured keratinocytes. Oncogene 25 : 2601-2614, 2006
13. Duthie MS, Kimber I, Norval M : The effect of ultraviolet radiation on the human immune system. Br J Dermatol 140 : 995-1009, 1999
14. Morita K, Saito T, Ohta M, Ohmori T, Kawai K, Teshima-Kondo S, Rokutan K : Expression analysis of psychological stress-associated genes in peripheral blood leukocytes. Neurosci Lett 381 : 57-62, 2005
15. Kawai T, Morita K, Masuda K, Nishida K, Shikishima M, Ohta M, Saito T, Rokutan K : Gene expression signature in peripheral blood cells from medical students exposed to chronic psychological stress. Biol Psychol 76 : 147-155, 2007
16. Heck DE, Gerecke DR, Vetrano AM, Laskin JD, Solar ultraviolet radiation as a trigger of cell signal transduction. Toxicol Appl Pharmacol 195 : 288-297, 2004
17. Fan H, Hegde PS : The transcriptome in blood : challenges and solutions for robust expression profiling. Curr Mol Med 5 : 3-10, 2005
18. Feezor RJ, Baker HV, Mindrinos M, Hayden D, Tannahill CL, Brownstein BH, Fay A, MacMillan S, Laramie J, Xiao W, Moldawer LL, Cobb JP, Laudanski K, Miller-Graziano CL, Maier RV, Schoenfeld D, Davis RW, Tompkins RG : Whole blood and leukocyte RNA isolation for gene expression analyses. Physiol Genomics 19 : 247-254, 2004
19. Debey S, Schoenbeck U, Hellmich M, Gathof BS, Pillai R, Zander T, Schultze JL : Comparison of different isolation techniques prior gene expression profiling of blood derived cells : impact on physiological responses, on overall expression and the role of different cell types. Pharmacogenomics J 4 : 193-207, 2004
20. Thach DC, Lin B, Walter E, Kruzelock R, Rowley RK, Tibbetts C, Stenger DA : Assessment of two methods for handling blood in collection tubes with RNA stabilizing agent for surveillance of gene expression profiles with high density microarrays. J Immunol Methods 283 : 269-279, 2003
21. Sesto A, Navarro M, Burslem F, Jorcano JL : Analysis of the ultraviolet B response in primary human keratinocytes using oligonucleotide microarrays. Proc Natl Acad Sci USA 99 : 2965-2970, 2002
22. Enk CD, Shahar I, Amariglio N, Rechavi G, Kaminski N : Gene expression profiling of *in vivo* UVB-irradiated human epidermis. Photodermatol Photoimmunol Photomed 20 : 129-137, 2004
23. Pisarchik A, Wortsman J, Slominski A : A novel microarray to evaluate stress-related genes in skin : effect of ultraviolet light radiation. Gene 341 : 199-207, 2004
24. Murakami T, Fujimoto M, Ohtsuki M, Nakagawa H : Expression profiling of cancer-related genes in human keratinocytes following non-lethal ultraviolet B irradiation. J Dermatol Sci 27 : 121-129, 2001
25. Garssen J, van Loveren H : Effects of ultraviolet exposure on the immune system. Crit Rev Immunol 21 : 359-397, 2001
26. Dröge W : Free radicals in the physiological control of cell Function. Physiol Rev 82 : 47-95, 2002
27. Karin M, Cao Y, Greten FR, Li ZW : NF-κB in cancer : from innocent bystander to major culprit. Nat Rev Cancer 2 : 301-310, 2002
28. Morison WL, Parrish JA, Bloch KJ, Krugler JI : *In vivo* effect of UVB on lymphocyte function. Br J Dermatol 101 : 513-519, 1979
29. Wiggin GR, Soloaga A, Foster JM, Murray-Tait V, Cohen P, Arthur JSC : MSK1 and MSK2 are required for the mitogen- and stress-induced phosphorylation of CREB and ATF1 in fibroblasts. Mol Cell Biol 22 : 2871-2881, 2002
30. Chen YH, Yang CK, Xia M, Ou CY, Stallcup MR : Role of GAC63 in transcriptional activation mediated by β-catenin. Nucl Acids Res 35 : 2084-2092, 2007