Genomics & Informatics Vol. 3(2) 66-72, June 2005

Aging and UV Irradiation Related Changes of Gene Expression in Primary Human Keratinocytes

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Abstract

The epidermis is a physiological barrier to protect organisms against environment. During the aging process, skin tissues undergo various changes including morphological and functional changes. The transcriptional regulation of genes is part of cellular reaction of aging process. In order to examine the changes of gene expression during the aging process, we used the primary cell culture system of human keratinocytes. Since UV radiation is the most important environmental skin aggressor, causing skin cancer and other problems including premature skin aging, we examined the changes of gene expression in human keratinocytes after UV irradiation using oligonucleotide microarray containing over 10,000 genes. We also compared the gene expression patterns of the senescent and UV treated cells. Expression of the variety of genes related to transcription factors, cell cycle regulation, immune response was altered in human keratinocytes. Some of down-regulated genes are represented in both senescent and UV treated cells. The results may provide a new view of gene expression following UVB exposure and aging process in human keratinocytes.

Keywords: keratinocyte, UVB irradiation, aging and microarray

Introduction

Normal human skin is covered by a multi-layered epidermis in which keratinocytes (KCs) undergo a continuous process of proliferation, differentiation and apoptosis (Funchs, 1990; Dotto, 1998). Skin is responsible for protecting our internal organs from the toxic external world. Skin protects us from heat, cold, and physical injuries. It also provides us with sensory information about the nature of the external world and is our first defense against invasion by bacteria, viruses, and other toxic elements. The skin is also an excretory organ, removing toxins from the body via perspiration.

Skin aging is characterized by various cellular and morphological changes (West, 1994; Yaar *et al.*, 2002) that come with functional impairments such as slow wound healing (Gerstein *et al.*, 1993), decreased protection against ultraviolet radiations (Gilchrest *et al.*, 1982), and increased susceptibility to skin cancer (Wei, 1998). Most of these alterations can be related to skin senescence, which entails among others: reduced proliferation (Gerstein *et al.*, 1993), increased oxidative stress (Chen, 2000) and reduced DNA repair capacity (Moriwaki *et al.*, 2000). Cellular senescence, initially observed during subculturing of normal diploid fibroblasts, can also be induced by hyperactivation of mitogenic pathways (Serrano *et al.*, 1997), chronic exposure to cellular stress, such as UV light, oxidative stress (Chen and Ames, 1994), or DNA damaging agents (Chang *et al.*, 1999). This "premature" senescence is indistinguishable from cellular senescence induced by serial passaging of human cells, suggesting that cellular senescence represents a fate choice influenced by extracellular and intracellular signal transduction pathways.

At the molecular level, cellular senescence is controlled by the tumor suppressor protein pRb and p53 (Dimri and Campisi, 1995; Campissi, 2001). Inactivation of these tumor suppressors results in bypass of senescence. Due to its essentially irreversible growth arrest and the requirement for p53 and pRb function, cellular senescence is considered a potent tumor suppressor mechanism.

UV radiation is the most important physical carcinogen in the environment, and the skin is its main target. UV irradiation, especially that induced by UVB (290-320 nm), causes the initiation and promotion of skin cancers, photo-aging, and immune suppression (Kripke, 1984; Streilein *et al.*, 1994; Fisher *et al.*, 1997). UVB has been thought to play a crucial role in the photo-carcinogenesis of epidermal keratinocytes, given that it introduces various types of damage to cellular DNA. One of the earliest detectable responses of human skin cells to UV irradiation is activation of multiple cytokine and growth factor cell surface receptors, including epidermal growth

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Accepted 15 March 2005

factor receptor (EGF-R; Sachsenmaier *et al.*, 1994; Warmuth *et al.*, 1994; Fisher and Voorhees, 1998), tumor necrosis factor (TNF)- α receptor (Dy *et al.*, 1999), platelet-activating factor (PAF) receptor (Dy *et al.*, 1999), insulin receptor (Coffer *et al.*, 1995), interleukin (IL)-1 receptor (Rosette and Karin, 1996), and platelet derived growth factor (PDGF) receptor (Knebel *et al.*, 1996).

More specifically, it is proposed that UV activation of membrane receptors stimulates MAP kinases signal transduction pathways, via stimulation of GTP-binging proteins including ras, rac, and cdc42. In support of this, activation of the three MAP kinases, ERK, Jnk, and p38 within 1hour of acute UV radiation has been demonstrated (Fisher *et al.*, 1998). This activation is then succeeded by increased expression of the transcription factors c-jun and c-fos that, together with other protein factors form the transcription factor complex AP-1 (Karin and Hunter, 1995). Elevated levels of AP-1 are then responsible for inducing expression of key members of the MMP family responsible for the degradation of dermal matrix.

To understand the mechanisms of UV response during the aging process, we used oligonucleotide microarray technology to determine changes in gene expression in human keratinocytes after UVB treatment. Cultured human epidermal keratinocytes were treated with 40 mJ/cm^2 UVB, and samples were collected at 4 hours after UVB treatment. Also senescent cells were collected at the same time. Oligonucleotide microarray containing over 10,000 genes were used to quantitatively assess changes in gene expression. Some of regulated genes are represented both of the senescent and UV treated cells.

Materials and Methods

Cell culture

Primary human keratinocytes from foreskin were maintained in keratinocyte growth medium (KGM) supplemented with bovine pituitary extract, human epidermal growth factor, insulin, hydrocortisone, gentamicin, and amphotericin B (Biowhitaker). The culture were kept at 37℃ in a 5% CO_2.

UV irradiation

For irradiation, the medium was removed and the keratinocytes treated with 40 mJ/cm^2 UVB. After irradiation, keratinocytes were rinsed with PBS and were placed in fresh medium. The cells maintained in culture for 4 hours. The source of UVB irradiation was a PHILIPS TL20 w/01 RS.

RNA extraction and array hybridization

Total RNA was isolated by using TRIzol reagent (Life Technologies, Inc.) following the manufacturers instruction. RNA was amplified and labeled with Cy3 or Cy5. Samples were hybridized to human 10k oligo chip (Macrogen). Prehybridization using hybridization buffer containing 4×SSC, 0.5% SDS, 0.5% BSA was performed at 42℃ for 1 hour. Prepared probe was hybridized with chip at 42℃ for 24 hours. After hybridization, chip was washed at room temperature for 5 minutes each in solution of wash buffer I (2× SSC/0.1% SDS), wash buffer II (1× SSC), wash buffer III (0.5% SSC), and then dried by air blower. Images were scanned by using Amersham Pharmacia Generation III scanner.

Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was performed with a Superscipt pre-amplification system (Life Technologies, Inc.). PCR was performed as follows: first, a denaturation step of 95℃ for 5 minutes; second, 95℃ for 1 minute; third, each annealing temperature, for 1 minute; fourth, 72℃ for 1 minute; fifth, 72℃ for 10 minutes. Amplification was performed by 27 cycles of second to fourth steps.

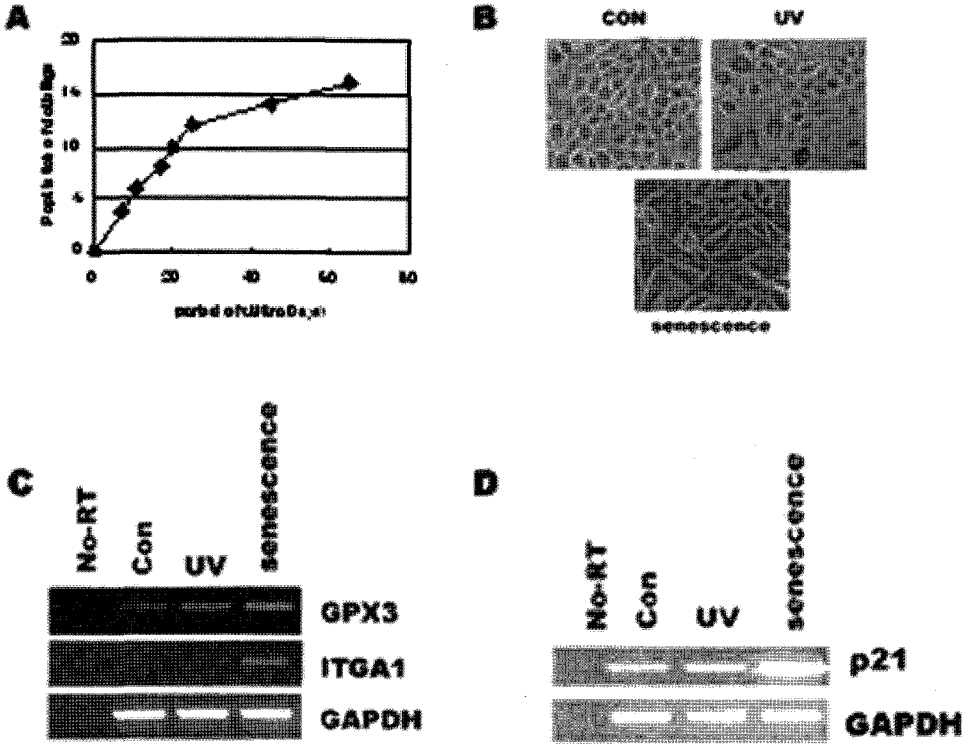
SA β-galactosidase assay

Detection of SA-• -galactosidase (pH 6.0) was performed as described by Dimri *et al.* (1995). Cells were washed in PBS and fixed for 5 minutes in 3% formaldehyde, resulting cells were washed and incubated 37℃ with SA-• -Gal staining solution (1 mg of 5-bromo-4-chloro-3-indolyl-• -D-galactoside/ml (Amersharm life science), 40 mM citric acid/sodium phosphate, pH 6.0, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 150 mM NaCl, 2 mM $MgCl_2$).

Staining was evaluated after 24 hours incubation at 37℃ in CO_2 . free incubater. The stained cells were visualized under microscope.

Analysis of image data

Imagene 4.0 (BioDiscovery) was used to analyze image data. A three-fold difference in signal intensity between the test and control values was considered to represent a significant difference in gene expression following UVB irradiation and in that of senescence.



Fig, 1. Replication kinetics of serially subcultured primary keratinocytes.
(A) The growth curve of primary human keratinocytes. Primary keratinocytes were serially passaged until the cells stopped dividing. Population doubling (PD) was measured and plotted against culture period. SA β-Gal activity (B), senescence- associated gene (GPX3, ITGA1) and p21 expression (C, D) the exponential cells, UV-treat cells and senescent cells.

Results

Life span and replicative senescence of a primary human keratinocytes

Primary keratinocytes were serially subcultured as dispersed cells in KGM until the cells reached the senescent phase (Fig. 1A). Population doubling (PD) was measured as a serial cultivation and the split ratio of 1:4 was performed. The growth of the primary keratinocytes slowed down around 12 PD and ceased at 16 PD (Fig. 1A).

Morphological change at senescent keratinocytes through microscopic observation was similar to the result previously observed (Fig. 1B). The senescent cells showed a flattened morphology, perinuclear vacuolization, increased cytoplasmic to nuclear ratio, and SA β-Gal expression, which are features of senescence cells previously described for primary keratinocytes (Bernard *et al.*, 2004). In contrast, the exponential phase cells showed undifferentiated keratinocyte cell morphology and did not express detectable SA β-Gal activity. Expression of the genes involved in the senescencing process and p21 as previously described was identified by RT-PCR (Fig. 1C, D).

Microarray analysis to determine UVB-regulated genes in primary human keratinocytes

Oligonucleotide microarray analysis of 10,000 human genes was performed to reveal genes up-regulated or down-regulated by at least three-fold after UVB-exposure. The keratinocytes received 40 mJ/cm^2 UVB irradiation. RNA was extracted 4 hours after UVB treatment for comparative hybridization experiments. 63 genes

Table 1. Functional classification of UVB-regulated genes.

Function	Increased	Decreased
Adhesion	6	4
Apoptosis	3	1
Cell cycle	14	5
Immune response	3	1
Metabolism	4	10
Signaling	5	12
Stress response	2	1
Transferase activity	2	7
Transcription	4	14
Transport	5	10
Others	15	17
Total	63	82

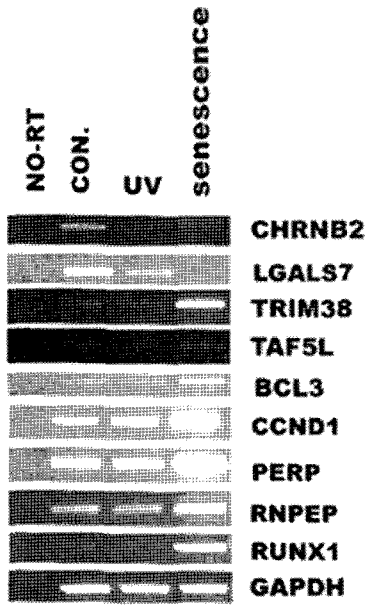


Fig. 2. Confirmation of microarray results by RT-PCR.
RNAs were prepared from keratinocytes with the indicated dose either 4 hours after exposure to UVB and senescent cells. The senescent cells were obtained from serially subcultured until population doubling (PD) of 12. GAPDH was used as a loading control.

appeared to be up-regulated by at least three-fold (Table 2), and 82 genes appeared to be down-regulated by at least three-fold (Table 3).

The differentially expressed genes identified in this assay have been reported to function in a variety of cellular processes including cell adhesion, cell cycle, signaling, immune response, transport and other cellular functions (Table 1). Several genes are worthy of note due to their association with UVB irradiation and their known roles in cellular regulatory pathways that have the potential to be influenced by UVB irradiation. Some of those identified by the microarray were validated through RT-PCR (Fig. 2). Furthermore, we investigated expression of some of those at 12 hours, 24 hours after irradiation and in senescence, respectively (Fig. 3B).

SA β-Gal assays, based on the formation of a blue precipitate due to 5-bromo-4-chloro-3-indolyl-β-D-galacto-pyranoside cleavage at pH 6.0, were performed on young keratinocytes and young keratinocytes incubated at 4 hours, 12 hours and 24 hours after irradiation, as well as in senescent keratinocytes. Numerous large cells that had accumulated the blue precipitate were found in the population of both UV-treated cells and normal senescent

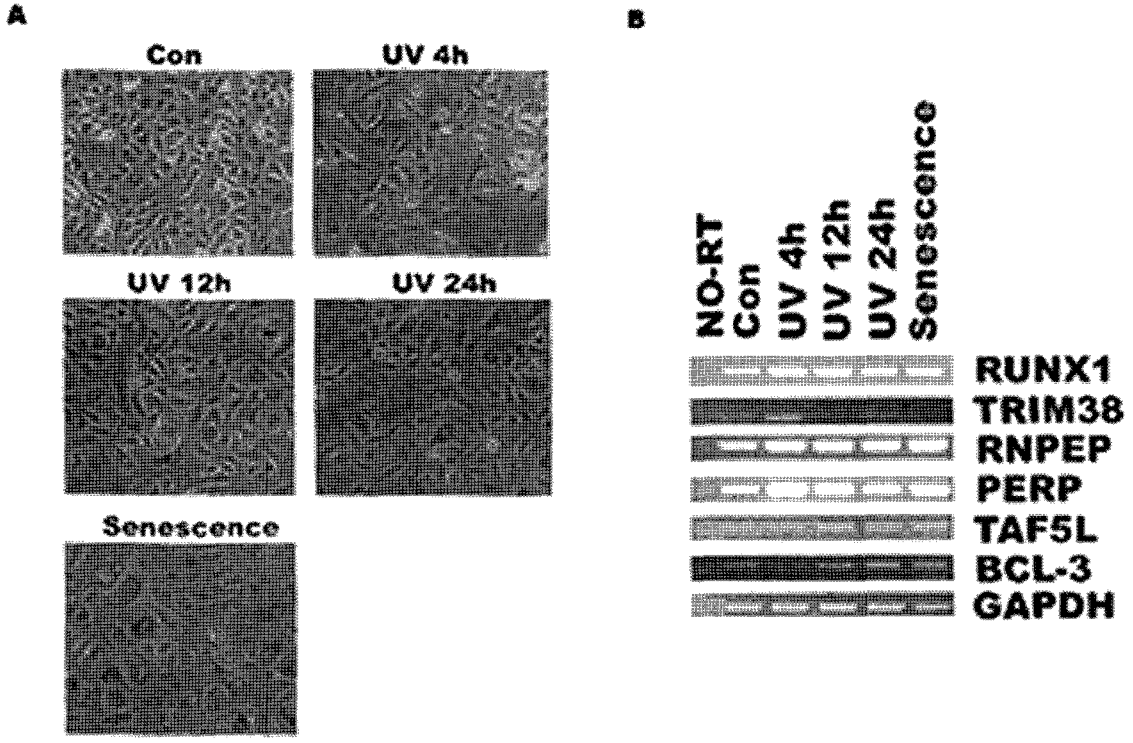


Fig. 3. SA-β-Gal activity and gene expression in UV-irradiated keratinocytes and senescent keratinocytes.
(A) SA-β-Gal assays were performed in young keratinocytes, 4 hours and 12 hours, 24 hours after UV-irradiation, or in senescent keratinocytes. The accumulation of a dark green precipitate, representative of SA-β-Gal activity, is observable in UV-irradiated cells as well as in normal senescent cells. (B) RNAs were prepared from keratinocytes irradiated at 40mJ/cm^2 in 4 hours, 12 hours and 24 hours after exposure to UVB and senescent cells. GAPDH was used as a loading control.

Table 2. Up-regulated genes expression affer UV irradiation.

Gene ID[a]	Fold induction[b]	Symbol	Gene Description
U40870	37.94	IGF-I	insulin-like growth factor-I
NM_002686	34.21	PNMT	phenylethanolamine N-methyltransferase
NM_017572	28.52	MKNK2	MAP kinase interacting serine/threonine kinase 2
AF343725	28.06	GPR54	G-protein-coupled receptor GPR54
NM_005824	25.29	LRRC17	leucine rich repeat containing 17
S67659	20.73	CANX	calnexin
AF174600	16.93	FBX20	F-box protein Fbx20
NM_002487	16.64	NDN	necdin
NM_020646	16.54	ASCL3	achaete-scute complex (Drosophila) homolog-like 3
AK024446	14.9	ABCC10	ATP-binding cassette,subfamily C(CFTR/MRP), member 10
NM_006625	13.24	FUSIP1	FUS interacting protein (serine-arginine rich) 1
NM_014449	10.79	GRCA	gene rich cluster, A gene
NM_013289	10.12	KIR3DL1	killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 1
NM_003827	9.45	NAPA	N-ethylmaleimide-sensitive factor attachment protein, alpha
NM_003300	9.39	TRAF3	TNF receptor-associated factor 3
NM_004507	9.23	HUS1	HUS1 checkpoint homolog (S. pombe)
NM_021219	8.67	JAM2	junctional adhesion molecule 2
NM_018479	8.54	ECHDC1	enoyl Coenzyme A hydratase domain containing 1
NM_003389	8.42	CORO2A	coronin, actin binding protein, 2A
NM_007002	8.24	ADRM1	adhesion regulating molecule 1
Z97068	8.2	CYR61	alternative spliced Cyr61 protein
NM_004620	6.85	TRAF6	TNF receptor-associated factor 6
NM_001549	6.77	IFIT3	interferon-induced protein with tetratricopeptide repeats 3
NM_004998	6.75	MYO1E	myosin IE
NM_005655	6.55	TIEG	TGFB inducible early growth response
NM_004670	6.24	PAPSS2	3'-phosphoadenosine 5'-phosphosulfate synthase 2
NM_000680	6.09	ADRA1A	adrenergic, alpha-1A-, receptor
NM_016233	6	PADI3	peptidyl arginine deiminase, type III
NM_002646	5.95	PIK3C2B	phosphoinositide-3-kinase, class 2, beta polypeptide
NM_018492	5.85	TOPK	T-LAK cell-originated protein kinase
NM_005778	5.5	RBM5	RNA binding motif protein 5
BC005068	5.36	A1-5	zinc finger protein 2
NM_012127	5.21	CIZ1	CDKN1A interacting zinc finger protein 1
NM_001489	5.15	NR6A1	nuclear receptor subfamily 6, group A, member 1
NM_004396	5.13	DDX5	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5
NM_002989	5.12	CCL21	chemokine (C-C motif) ligand 21
NM_007254	5	PNKP	polynucleotide kinase 3'-phosphatase
U38980	4.93	hPMSR6	PMS2 related
NM_016101	4.88	CGI-37	comparative gene identification transcript 37
NM_005836	4.68	HRSP12	heat-responsive protein 12
AF118224	4.5	ST14	matriptase
NM_014622	4.45	LOH11CR2A	loss of heterozygosity, 11, chromosomal region 2, gene A
NM_004603	4.34	STX1A	syntaxin 1A (brain)
NM_018685	4.26	ANLN	anillin, actin binding protein (scraps homolog, Drosophila)
NM_018903	3.89	PCDHA12	protocadherin alpha 12
NM_001996	3.76	FBLN1	fibulin 1
AL589867	3.66	TTLL1	tubulin tyrosine ligase-like family, member 1
AB047004	3.62	PC-LKC	protocadherin LKC
NM_016542	3.61	MST4	Mst3 and SOK1-related kinase
NM_001932	3.58	MPP3	membrane protein, palmitoylated 3 (MAGUK p55 subfamily, member 3)
AL122063	3.49	GLTSCR2	glioma tumor suppressor candidate region gene 2
NM_020410	3.48	ATP13A	ATPase type 13A
NM_004997	3.25	MYBPH	myosin binding protein H
AF191687	3.23	TLH6	alanine-glyoxylate aminotransferase homolog
NM_004467	3.23	FGL1	fibrinogen-like 1
NM_002011	3.22	FGFR4	fibroblast growth factor receptor 4
NM_016457	3.2	PRKD2	protein kinase D2
NM_018437	3.18	HEMGN	hemogen
AJ249366	3.16	COPE	coatomer protein complex, subunit epsilon
AF2970158 1	3.12	RGS8	regulator of G-protein signalling 8
NM_006442	3.05	DRAP1	DR1-associated protein 1 (negative cofactor 2 alpha)
NM_016735	3.05	LIMK1	LIM domain kinase 1
NM_014207	3.04	CD5	CD5 antigen (p56-62)

[a] Genbank accession number.
[b] Ratio between gene expression levels in control human keratinocytes and UVB- treated keratinocytes.

Table 3. Down-regulated genes expression affer UV irradiation.

Gene ID[a]	Fold induction[b]	Symbol	Gene Description
NM_005178	-4.98	BCL3	B-cell CLL/lymphoma 3
NM_006355	-4.93	TRIM38	tripartite motif-containing 38
AF077186	-4.9	CHRNB2	neuronal nicotinic acetylcholine receptor beta 2
NM_014409	-4.7	TAF5L	TAF5-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor, 65kDa
AK027344	-4.58	FIBL-6	hemicentin
NM_000432	-4.54	MYL2	myosin, light polypeptide 2, regulatory, cardiac, slow
NM_006564	-4.43	CXCR6	chemokine (C-X-C motif) receptor 6
NM_005080	-4.41	XBP1	X-box binding protein 1
NM_020216	-4.4	RNPEP	arginyl aminopeptidase (aminopeptidase B)
NM_001405	-4.39	EFNA2	ephrin-A2
NM_020167	-4.34	NMUR2	neuromedin U receptor 2
NM_014187	-4.33	HSPC171	HSPC171 protein
BC000019	-4.25	CDH6	cadherin 6, type 2, K-cadherin (fetal kidney)
AF222903	-4.22	PPY2	pancreatic polypeptide-2
NM_000149	-4.17	FUT3	fucosyltransferase 3 (galactoside 3(4)-L-fucosyltransferase, Lewis blood group included)
NM_001758	-4.16	CCND1	cyclin D1 (PRAD1: parathyroid adenomatosis 1)
NM_004973	-4.11	JARID2	Jumonji, AT rich interactive domain 2
AF324466	-4.07	C22orf23	chromosome 22 open reading frame 23
AF134979	-4.05	NPCR	nasopharyngeal carcinoma-related protein
NM_016423	-4.04	ZNF219	zinc finger protein 219
NM_006285	-4.04	TESK1	testis-specific kinase 1
AF301906	-4.03	FOXL2	forkhead transcription factor FOXL2
NM_016818	-4.03	ABCG1	ATP-binding cassette, sub-family G (WHITE), member 1
U78556	-4.03	hCRA alpha	cisplatin resistance associated alpha protein
AJ001898	-4.02	GDNF	glial cell derived neurotropic factor
AF000996	-4	UTY	ubiquitous TPR motif, Y isoform
NM_015680	-4	C2orf24	chromosome 2 open reading frame 24
NM_033440	-3.98	ELA2A	elastase 2A
NM_005235	-3.91	ERBB4	v-erb-a erythroblastic leukemia viral oncogene homolog 4 (avian)
NM_032009	-3.9	PCDHGA2	protocadherin gamma subfamily A, 2
NM_022121	-3.9	PERP	TP53 apoptosis effector
NM_012413	-3.86	QPCT	glutaminyl-peptide cyclotransferase (glutaminyl cyclase)
NM_001332	-3.85	CTNND2	catenin (cadherin-associated protein), delta 2 (neural plakophilin-related arm-repeat protein)
NM_007155	-3.85	ZP3	zona pellucida glycoprotein 3 (sperm receptor)
AF380932	-3.81	MYO1G	unconventional myosin 1G valine form
AF260332	-3.78	C1QA	complement component,q subcomponent, alpha polypeptide
NM_020310	-3.76	MNT	MAX binding protein
AF387103	-3.76	PASK	PAS-kinase
NM_005266	-3.75	GJA5	gap junction protein, alpha 5, 40kDa (connexin 40)
NM_006744	-3.73	RBP4	retinol binding protein 4, plasma
AY048119	-3.73	RASGRP4	RAS guanyl releasing protein 4
AL096750	-3.73	OPLAH	5-oxoprolinase(ATP-hydrolysing)
AF298591	-3.72	NHE7	nonselective sodium potassium/proton exchanger
U80987	-3.72	TBX5	T-box 5
NM_004210	-3.68	NEURL	neuralized-like (Drosophila)
NM_057090	-3.67	ARTN	artemin
NM_000201	-3.65	ICAM1	intercellular adhesion molecule 1 (CD54), human rhinovirus receptor
NM_006812	-3.64	OS-9	amplified in osteosarcoma
NM_006186	-3.63	NR4A2	nuclear receptor subfamily 4, group A, member 2
NM_001610	-3.62	ACP2	acid phosphatase 2, lysosomal
NM_007017	-3.62	SOX30	SRY (sex determining region Y)-box 30
NM_004772	-3.61	C5orf13	chromosome 5 open reading frame 13
NM_002083	-3.61	GPX2	glutathione peroxidase 2 (gastrointestinal)
U17986	-3.6	SLC6A8	solute carrier family 6 (neurotransmitter transpoter, creatine), member 8
NM_001754	-3.6	RUNX1	runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)
NM_014617	-3.6	CRYGA	crystallin, gamma A
AF380833	-3.59	STN2	stonin 2
NM_021727	-3.59	FADS3	fatty acid desaturase 3
NM_002043	-3.58	GABRR2	gamma-aminobutyric acid (GABA) receptor, rho 2
NM_013360	-3.58	ZNF222	zinc finger protein 222
AK001830	-3.57	C15orf12	chromosome 15 open reading frame 12
NM_003644	-3.54	GAS7	growth arrest-specific 7
NM_001776	-3.53	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1
AJ000334	-3.53	NARS	asparaginyl-t RNA synthetase
NM_004594	-3.53	SLC9A5	solute carrier family 9 (sodium/hydrogen exchanger), isoform 5
NM_000459	-3.52	TEK	TEK tyrosine kinase, endothelial (venous malformations, multiple cutaneous and mucosal)
NM_020181	-3.5	C14orf162	chromosome 14 open reading frame 162
AY033290	-3.5	ABHD1	abhydrolase domain containing 1
NM_001538	-3.48	HSF4	heat shock transcription factor 4
NM_002479	-3.45	MYOG	myogenin (myogenic factor 4)
AJ243500	-3.45	TRPV6	transient receptor potential channel 6, subfamily V,
NM_020184	-3.44	CNNM4	cyclin M4
NM_020230	-3.4	PPAN	peter pan homolog (Drosophila)
D17390	-3.4	ADAM11	a disintegrin and metalloprotease domain 11
NM_014068	-3.39	PSORS1C1	psoriasis susceptibility 1 candidate 1
AB011539	-3.39	KIAA0815	MEGF6 protein
L24521	-3.38	HDGF	hepatoma-derived growth factor (high-mobility group protein 1-like)
AJ224867	-3.37	GNAS	GNAS complex locus
AJ295938	-3.37	SYBL1B	synaptobrevin like protein 1B
NM_030674	-3.37	SLC38A1	solute carrier family 38, member 1
NM_003595	-3.36	TPST2	tyrosylprotein sulfotransferase 2
NM_000287	-3.35	PEX6	peroxisomal biogenesis factor 6

[a] Genbank accession number.
[b] Ratio between gene expression levels in control human keratinocytes and UVB- treated keratinocytes.

cells, whereas young small control cells were negative (Fig. 3A). Therefore, we conclude that UV irradiation provokes premature senescence in young keratinocytes.

Discussion

As a biological barrier, the skin is continuously subjected to environmental stresses such as ultraviolet radiation, pathogens and thermal and chemical insults (Slominski and Wortsman, 2000). Ultraviolet (UV) radiation from the sun is among the most ubiquitous damaging environmental factors from which human skin must protect itself (Brunet *et al.*, 1999). UVB wavelengths are the most energetic,

and are responsible for sunburn. UVB is directly absorbed by DNA and protein, and as such account for much of the damaging biological effects of UV irradiation including cancer and premature skin aging (Fisher *et al.*, 1997).

To explore a role for UV irradiation in aging of skin, we used microarray technology and detected several genes with changed expression levels (up- or down-regulated) after exposure to UVB. As the results, 63 genes appeared to be up-regulated (Table 2), and 82 genes appeared to be down-regulated by at least three-fold (Table 3). Expression of the genes involved in metabolism, signaling, transcription, transport were suppressed, whereas cell cycle, immune response, cell adhesion were induced (Table 1). Some of gene expression was confirmed by RT-PCR, and then we compared gene expression in UVB-treated cells and the senescent cells (Fig. 2). Two genes of those, CHRNB2 and LGALS7 were decreased by UVB irradiation and senescence. The other genes were decreased by UVB irradiation, but increased in senescence. In previous study, LGALS7 (Galectin-7) was reported to be up-regulated in the human keratinocytes at 1 hour after UVB irradiation (Bernerd *et al.*, 1999). However, in our results, LGALS7 was down- regulated in UVB-treated cells and the senescent cells. LGALS7 has diverse biological functions, including regulation of cell adhesion, cell growth and apoptosis (Kasai and Hirabayashi, 1996: Perillo *et al.*, 1998; Cooper and Barondes, 1999; Liu, 2000). In recent study, LGALS7 exhibits pro-apoptotic function (Ichiro *et al.*, 2002). However, our data demonstrate that UVB (40 mJ/cm^2) irradiation did not activate apoptotic pathway.

Bcl-3 (B cell CLL/lymphoma 3) is a proto-oncogene candidate. It is identified by its translocation into the immunoglobulin alpha-locus in some cases of B-cell leukemia (Franzoso *et al.*, 1992). This protein functions as a transcriptional co-activator that activates through its association with NF-kappa B homodimers (Bours *et al.*, 1993: Fujita *et al.*, 1993). The expression of this gene can be induced by NF-kappa B, which forms a part of the autoregulatory loop that controls the nuclear residence of p50 NF-kappa B (Brasier *et al.*, 2001). p53 represses cyclin D1 transcription through down regulation of Bcl-3 protein levels. UV irradiation also leads to the repression of cyclin D1and Bcl-3 (Rocha *et al.*, 2003). In our study, the expression of Bcl-3 was decreased in UVB- treated cells, which was consistent with the previous study. However, in senescent cells, the expression of Bcl-3 was increased differently from that of UVB- treated cells in our study.

We also compared SA β-Gal activity of young keratinocytes, UV-treated keratinocytes and senescent keratinocytes. Numerous large cells that had accumulated the blue precipitate were found in the population of both UV-treated cells and normal senescent cells, whereas young control cells were negative (Fig. 1B and 3A).

These finding combined with our results support that the keratinocytes after UV irradiation enter the initial senescent phase and the possibility that UVB-regulated genes play an important role in skin aging.

Acknowledgements

This study was supported by Grant 02-PJ10-PG6-AG01-0003 from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea

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