

cDNA Microarray Analysis of Gene Expression Profiles in Human Fibroblast Cells Irradiated with Red Light

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In this paper, the cDNA microarray technique was used to investigate the gene expression profiles of human fibroblasts irradiated by low-intensity red light. Proliferation assays showed that the fibroblast HS27 cells responded with a curve effect to different doses of low-intensity red light irradiation at a wavelength of 628 nm. An optimal dose of 0.88 J per cm² was chosen for subsequent cDNA microarray experiments. The gene expression profiles revealed that 111 genes were regulated by the red light irradiation and can be grouped into 10 functional categories. Most of these genes directly or indirectly play roles in the enhancement of cell proliferation and the suppression of apoptosis. Two

signaling pathways, the p38 mitogen-activated protein kinase signaling pathway and the platelet-derived growth factor signaling pathway, were found to be involved in cell growth induced by irradiation of low-intensity red light. Several genes related to antioxidation and mitochondria energy metabolism were also found to express differentially upon irradiation. This study provides insight into the molecular mechanisms associated with the beneficial effects of red light irradiation in accelerating wound healing. **Key words:** cDNA microarray/gene expression profiles/human fibroblast/red light/wound healing. *J Invest Dermatol* 120:849–857, 2003

Low-intensity red laser light, one of the red light sources, was first used to accelerate wound healing in the 1970s (Mester *et al*, 1971). This phototherapy has attracted increasing attention in recent years because numerous applications and controlled studies on the method have been reported. Low-intensity red laser light therapy has been applied to various medical conditions such as wound repair and dermatologic diseases, neurologic damage, blood disorders, musculoskeletal complications, and pain and inflammation (Abergel *et al*, 1984; Mester *et al*, 1985; Lyons *et al*, 1987; Rochkind and Ouaknine, 1992; Conlan *et al*, 1996; Lee *et al*, 1996; Wedlock *et al*, 1996; Yaakobi *et al*, 1996; Schindl *et al*, 1998; 2000; Reddy *et al*, 2001). Previous studies showed that low-intensity red laser light was capable of affecting cellular processes in the absence of significant thermal effects (Karu, 1989). Fibroblasts are an important cellular component of wound healing, and it has been reported that fibroblasts grow faster than control after exposure to red light in suitable doses (Boulton and Marshall, 1986; Nara *et al*, 1992; Lubart *et al*, 1993; Yu *et al*, 1994a; Conlan *et al*, 1996; Schindl *et al*, 2000). Red light irradiation has also been reported to enhance the activity of succinic dehydrogenase and promote the synthesis of procollagen and the release of basic fibroblast growth factor (Conlan *et al*, 1996; Yu *et al*, 1994a; Bolton *et al*, 1995; Yamamoto *et al*, 1996). Although the evidence of the beneficial effects of low-intensity red light therapy is mounting, the

molecular mechanisms of biomodulation by the irradiation remain poorly understood.

The commonly used low-intensity red light source is the He–Ne laser, which emits red light at a wavelength of 632.8 nm. The laser is frequently replaced by low-cost noncoherent diodes. Previous cell culture studies showed that there was no difference in the biologic response to irradiation with coherent red laser light and noncoherent red light (Karu, 1987; Young *et al*, 1989). In this study, the effects of red light irradiation on cell proliferation were studied by irradiating human fibroblasts using low-intensity red-light-emitting diodes at wavelength 628 nm. An optimal dose of irradiation was first determined, and the gene expression profiles of human fibroblasts upon irradiation were examined by using cDNA microarrays containing 9982 human genes and expressed sequence tags. Functional analysis of the differentially expressed genes was carried out in order to understand how low-intensity red light modulates the behavior of fibroblasts at the molecular level.

MATERIALS AND METHODS

Red light source An instrument equipped with red-light-emitting diodes (Nereid™ Red Light Phototherapy System, Healthtech, Shanghai, China) was used in this study. The diodes consist of four elements of InGaAlP and emit light at 628 nm (dominant wavelength). The irradiation distance is 0.75 cm from the top of the light source to the button of cell culture plates. There is a round window on the top of the red light equipment for irradiation. The diameter of the window is adjustable and suitable for tissue culture plates of 14, 9, or 3.5 cm diameter. Therefore, three irradiation areas, 153.8, 63.6, and 9.6 cm², are available from the red light equipment. The actually irradiated area was 9.6 cm² for cell proliferation assays and 153.8 cm² for gene expression profiling, as a greater amount of cells is required for the extraction of sufficient RNA for high-density cDNA microarray experiments. The power output was

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Abbreviation: MAPK, mitogen-activated protein kinase.

15 mW and the intensity of irradiation was 11.46 mW per cm² on the surface of the tissue culture plates at the irradiation distance of 0.75 cm. Samples were irradiated for various time periods to yield final energy doses of 0, 0.44, 0.88, 2.00, 4.40, and 8.68 J per cm². The temperature was monitored during irradiation and no detectable differences were found between treatments.

Cell culture and proliferation assay Normal human fibroblasts of HS27 newborn foreskin (ATCC, VA) were cultured at 37°C in a humidified atmosphere containing 5% CO₂, with Dulbecco's minimal essential medium supplemented with 5% fetal bovine serum, 100 units penicillin, 100 µg streptomycin, and 2 mM glutamine (all from Gibco BRL, CA). The cells were irradiated daily with the red-light-emitting diodes at wavelength 628 nm and different doses (0, 0.44, 0.88, 2, 4.4, and 8.68 J per cm²) for 3 d and the cell numbers were counted at day 3. The cells responded with a curve effect to different doses of low-intensity red light irradiation. The optimal dose to stimulate HS27 cell proliferation is 0.88 J per cm². This dose was chosen to treat cells for further cDNA microarray experiments.

Preparation of cDNA microarrays A cDNA library containing 9982 human genes and expressed sequence tags (Unigen Human ClonEST Version 2.0, Incyte Genomics, CA) was used for the fabrication of cDNA microarrays based on published protocols (Hegde *et al*, 2000). Briefly, the clones were subcultured in 96 well format using LB broth supplemented with 15% glycerol at 37°C overnight and were stored at -80°C with proper sealing. All the clones were amplified using universal primers and the polymerase chain reaction (PCR) products were used as probes on the DNA microarray. The PCR products were purified by isopropanol precipitation. The dried DNA pellet was resuspended using 1 × TE buffer with 15% glycerol. The quality and quantity of PCR products were checked by agarose gel electrophoresis. After standardizing the concentration of all PCR products by dilution, the PCR products were spotted on poly lysine treated glass slides using a spotter (SPBIO, Hitachi). The microarrays were baked at 80°C for 1 h, rehydrated, treated with a blocking solution (containing succinic anhydride, 1-methyl-2-pyrrolidinone, and sodium borate), and finally cross-linked with an ultraviolet cross-linker.

RNA isolation The fibroblast cells were cultured in 14 cm tissue culture plates and irradiated daily at an energy dose of 0.88 J per cm² for 3 d. The cells were allowed to culture for 8 h after the last irradiation. The cells were collected and total RNA was isolated with Trizol reagent (Gibco BRL, CA) according to the manufacturer's instruction. Total RNA concentrations were determined by their absorbance at 260 nm. The quality of RNA samples was verified by examining the integrity of 28S and 18S rRNA using 1% agarose gel electrophoresis. The total RNA from untreated cells was used as control.

cDNA microarray hybridization One hundred and fifty micrograms of total RNA from control or irradiated samples was reverse transcribed to cDNA in the presence of reverse transcriptase (SuperScript II, Life Technologies, CA). During the reaction, two distinct fluorescent dyes, Cy3-dUTP and Cy5-dUTP (Amersham Pharmacia Biotech, NJ), were incorporated into the cDNA for the control and treated samples, respectively. The Cy3- and Cy5-labeled cDNA were purified using a Microcon 30 column (Microcon, MA) and mixed in a 1:1 ratio to 50 µl. The cDNA sample was mixed with a hybridization solution containing 2 µl of each of the following reagents: poly (dA) (8 mg per l), human Cot-1 DNA (4 mg per l), yeast tRNA (10 mg per l), 6 µl of 20 × sodium citrate/chloride buffer (SSC), and 0.4 µl of 10% sodium dodecyl sulfate (SDS). The mixture was then denatured at 100°C for 2 min and applied to a microarray slide. Hybridization was carried out at 65°C overnight. Following 10 min washing in 2 × SSC, 0.1% SDS at 37°C, and twice in 0.2 × SSC at room temperature, the slide was scanned with a confocal laser scanner (ScanArray 4000, GSI Lumonics, MA).

Analysis of microarray data The microarray results were analyzed using a program called ScanAnalyze (M. Eisen, Stanford University, <http://genome-www.stanford.edu>). Fluorescent images were gridded to locate the spot corresponding to each gene. Fluorescence and background intensities for both Cy3 and Cy5 wavelengths were extracted for data normalization and analysis. The raw data were filtered according to the following criteria: spots with small diameters (<120 µm), low signal intensity (<300 fluorescence intensity units), and low signal to noise ratio (<1.5) were discarded. To ensure the reproducibility of the microarray results, the experiment was repeated with newly extracted total RNA samples. Fluorescence ratios (Cy5/Cy3) were used to determine the relative level of gene expression. Genes showing a greater than 2-fold induction or

repression (Cy5/Cy3 ratios above 2 or below 0.5) were selected for further functional analysis. The cutoff value of 2-fold is conventionally used by other investigators (Barrans *et al*, 2001; Quackenbush, 2001).

RESULTS AND DISCUSSION

Effects of red light on human fibroblast proliferation The effects of red light irradiation on cells are mediated by many factors, including dose of exposure, cell culture conditions, and irradiation wavelength. A cell proliferation assay was first carried out to study the response of HS27 fibroblasts to different doses of low-intensity red light irradiation. The cells were irradiated daily with different energy doses of red light at 628 nm for 3 d and the changes in cell number were recorded. The HS27 fibroblasts responded to the irradiation dose as a nonlinear curve and the cells grew faster than the control at energy doses of 0.44, 0.88, and 2 J per cm², with an optimal proliferation effect at 0.88 J per cm² (Fig 1). When the energy dose was increased to over 4 J per cm², the cells grew at the same rate as or slightly slower rates than control cells. Previous studies have reported that irradiation using an He-Ne laser at 632.8 nm, 660 nm, and 670 nm modulated cell growth (Schindl *et al*, 1997;

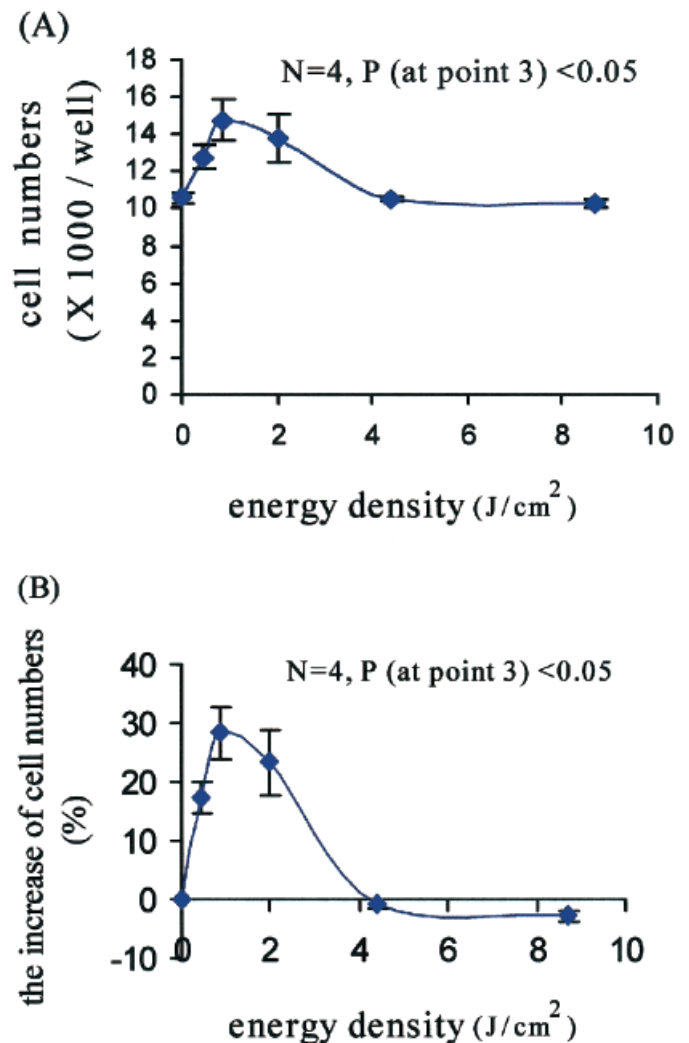


Figure 1. Effects of red light irradiation on cell proliferation. 3×10^4 HS27sk cells were placed in 3 cm plates and cultured using Dulbecco's modified essential medium with 5% fetal bovine serum. The cells were irradiated daily with red light at 628 nm wavelength, with an energy density of 0, 0.44, 0.88, 2.00, 4.40, or 8.68 J per cm² for 3 d. The cell numbers were counted at day 3. The cell numbers and the percentage increase in cell numbers with different treatments are shown in (A) and (B), respectively.

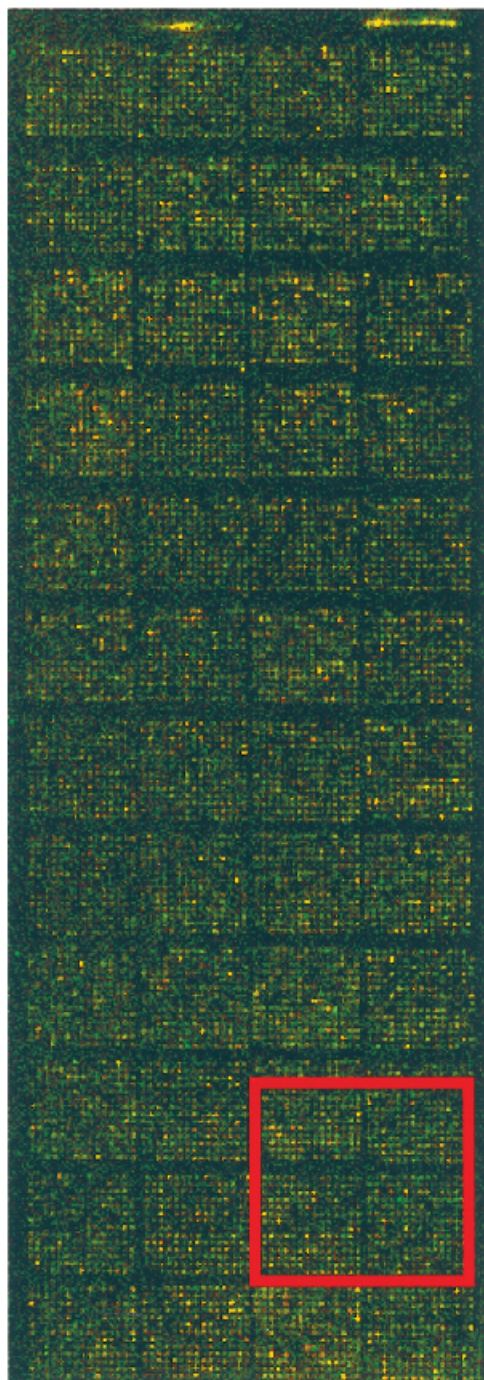
Stadler *et al.*, 2000; Pinheiro *et al.*, 2002). The above results demonstrated that low-intensity red light at 628 nm wavelength also enhanced cell proliferation.

Effects of red light on gene expression profile in human fibroblasts The gene expression profile of human fibroblasts irradiated with red light was obtained by hybridizing the fluorescein-labeled cDNA from human fibroblasts with and without red light irradiation with a cDNA microarray containing 9982 human genes and expressed sequence tags. A typical microarray image is shown in **Fig 2(A)** with Cy3 (*green*, control) and Cy5 (*red*, irradiated sample) fluorescence intensities overlapped in one image. An enlarged region of the microarray

image (**Fig 2B**) shows that the red spots correspond to an increased level of gene expression in cells irradiated with red light whereas the green spots correspond to a decreased level of gene expression in irradiated cells.

The experiment was carried out in duplicate and the average ratios of Cy5/Cy3 for the spots that survived the data filtering process were calculated and are summarized in **Fig 2(C)**. A total of 1143 spots survived the filtering process. Genes with a ratio of >2.0 or <0.5 were considered positively regulated or negatively regulated by red light, respectively. Using these criteria, we identified 111 genes that displayed differential expression upon red light irradiation (**Table I**). Among them, 68 genes were upregulated and 43 genes were downregulated. The result of a

(A)



(C) Summary of the microarray data

total genes	good spots	up regulation	down regulation
9982	1143	68	43

(D) Statistical analysis of the microarray data

Exp	mean ratio (Cy5/Cy3)	mean difference of ratio	d.f. (n - 1)	T value	P
1	1.13	0.14	1142	0.93	>0.05
2	0.99	—	—	—	—

(B)

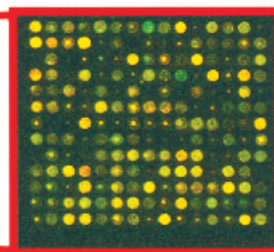


Figure 2. Expression profiling of HS27 newborn foreskin fibroblasts upon red light irradiation by using a 10,000 human cDNA microarray. (A) Microarray image overlapping Cy3 (control) and Cy5 (sample) fluorescence images. (B) An enlarged region of the microarray image. (C) Summary of the microarray data. (D) Statistical analysis of microarray data.

Table I. Differentially expressed genes of fibroblasts upon red light irradiation

Name of gene	Symbol	Ratio	Unigene ID
1. Proliferation			
<i>(stimulatory roles)</i>			
Cyclin-dependent kinase 5, regulatory subunit 1 (p35)	(CDK5R1)	4.7982	Hs.93597
Platelet derived growth factor C	(PDGFC)	4.0907	Hs.43080
Breakpoint cluster region	(BCR)	3.7040	Hs.234799
Spindle pole body protein	(DAG1)	3.0320	Hs.9884
Mitogen-activated protein kinase 11	(P38Beta2)	3.5626	Hs.57732
Serum response factor	(SRF)	2.1190	Hs.155321
Cyclin H	(CCNH)	0.4372	Hs.514
Kinesin-like 1	(KNSL1)	0.4310	Hs.8878
<i>(inhibitory role)</i>			
Cullin 1	(CUL1)	0.4599	Hs.14541
2. Antioxydant-related			
Selenoprotein W, 1	(SEPW1)	3.0693	Hs.14231
ATX1 antioxidant protein 1	(ATOX1)	2.0010	Hs.279910
3. Apoptosis and stress proteins			
<i>(enhancing apoptosis and stress)</i>			
Receptor (TNFRSF)-interacting serine-threonine kinase 1	(RIPK1)	3.9547	Hs.296327
Heat shock 70kD protein 1A	(HSPA1A)	0.4064	Hs.8997
Caspase 6	(CASP6)	0.2269	Hs.3280
Stress-induced-phosphoprotein 1	(STIP1)	0.1065	Hs.75612
<i>(supressing apoptosis and stress)</i>			
JAK binding protein,	(SSI-1)	3.9360	Hs.50640
4. Metabolism			
<i>(protein and amino acid)</i>			
Calnexin	(CANX)	3.4932	Hs.155560
Zinc metalloproteinase	(ZMPSTE24)	2.7458	Hs.25846
Branched chain aminotransferase 2	(BCAT2)	2.5200	Hs.101408
S-adenosylhomocysteine hydrolase	(AHCY)	2.4818	Hs.172673
Torsin family 1, member B (torsin B)	(TOR1B)	2.4551	Hs.252682
Proteasome subunit, beta type, 3	(PSMB3)	2.3828	Hs.82793
Peptidyl prolyl isomerase H (cyclophilin H)	(PIIH)	2.3340	Hs.9880
ELL-related RNA polymerase II	(ELL2)	0.3961	Hs.173334
Chaperonin containing TCP1 subunit 7 (eta)	(CCT2)	0.3642	Hs.6456
Peptidylglycine alpha-amidating monooxygenase	(PAMCI)	0.3516	Hs.159396
Vigilin	(HDLBP)	0.0345	Hs.177516
Alanyl (membrane) aminopeptidase	(ANPEP)	0.3003	Hs.1239
<i>(sugar)</i>			
Enolase 3 (beta, muscle)	(ENO3)	0.4054	Hs.118804
Aldolase A, fructose-bisphosphate	(ALDOA)	0.4814	Hs.273415
<i>(lipids)</i>			
Apolipoprotein C-III	(APOC3)	5.9013	Hs.73849
Lysophospholipase II	(LYPLA2)	4.4667	Hs.283655
Apolipoprotein AI regulatory protein-1,	(NR2F2)	0.3425	Hs.347991
<i>(energy metabolism and respiratory chain)</i>			
NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 2	(NDUFB2)	3.9493	Hs.198272
Electron-transfer-flavoprotein, beta polypeptide	(ETFB)	3.9360	Hs.74047
ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d	(ATP5H)	3.4880	Hs.64593
5. Ion channel, transport proteins and membrane potential			
ATP-binding cassette, sub-family A	(ABC1)	4.3787	Hs.211562
Potassium voltage-gated channel, subfamily G, member 1	(KCNG1)	3.8853	Hs.118695
Sodium channel, voltage-gated, type IV, alpha polypeptide	(SCN4A)	3.5733	Hs.46038
Potassium inwardly-rectifying channel subfamily J, member 13,	(KCNJ13)	2.0408	Hs.66726
Chloride intracellular channel 4	(CLIC4)	0.2461	Hs.25035
ArsA arsenite transporter, ATP-binding, homolog 1	(ASNA1)	0.2316	Hs.165439
6. Cytoskeleton, cell motility and extracellular matrix proteins			
<i>(cytoskeleton and motility)</i>			
Dystroglycan 1	(DAG1)	5.5333	Hs.76111
Ras homolog gene family, member D	(ARHD)	3.5787	Hs.15114

Table I. (continued)

Name of gene	Symbol	Ratio	Unigene ID
Myosin, heavy polypeptide 9	(MYH9)	3.0180	Hs.146550
RAN binding protein 9	(RANBP9)	2.3635	Hs.279886
Actin related protein 2/3 complex, subunit 5	(ARPC2)	0.4615	Hs.824250
Leucine rich repeat (in FLII) interacting protein 1	(LRRFIP1)	0.4452	Hs.326159
Tropomyosin 4	(TPM4)	0.3784	Hs.250641
Keratin, hair, acidic, 1	(KRTHA1)	0.1974	Hs.41696
(extracellular matrix)			
Fibromodulin	(FMOD)	3.0213	Hs.230
Tuftelin-interacting protein	(TIP39)	2.4377	Hs.20225
Fibrillin 1	(FBN1)	0.0460	Hs.750
Matrix metalloproteinase 10	(MMP10)	0.4121	Hs.2258
(migration, aggregation, and adhesion)			
Carcinoembryonic antigen-related cell adhesion molecule 3	(CEACAM3)	3.6747	Hs.11
Cadherin 12, type 2 (N-cadherin 2)	(CDH12)	3.6080	Hs.33399
Sphingosine-1-phosphatase	(OC81537)	2.3629	Hs.24678
Promotes cell adhesion	(ADRM1)	2.6090	Hs.90107
Cadherin 13, H-cadherin (heart)	(CDH13)	0.1839	Hs.63984
7. DNA and deoxyribonucleoside synthesis and repair			
N-methylpurine-DNA glycosylase	(MPG)	4.0187	Hs.79396
Adenine phosphoribosyltransferase	(APRT)	3.8587	Hs.28914
Nudix (nucleoside diphosphate linked moiety X)-type motif 1	(NUDT1)	2.5413	Hs.388
8. Transcription factors			
GCN5 general control of amino-acid synthesis 5-like 1	(GCN5L1)	4.5390	Hs.94672
Glioma-amplified sequence-41	(GAS41)	4.6187	Hs.4029
Putative zinc finger protein NY-REN-34 antigen	(LOC51131)	3.3653	Hs.279799
Zinc finger protein 74	(ZNF74)	0.3790	Hs.3057
Zinc finger protein 7	(ZNF7)	0.4401	Hs.2076
9. Immune/inflammation and cytokines			
Leukocyte receptor cluster (LRC) member 5	(LENG5)	2.1889	Hs.15580
Associated molecule with the SH3 domain of STAM	(AMSH)	2.6353	Hs.12479
Translin	(TSN)	0.4240	Hs.75066
Septin 6	(SEP2)	0.3125	Hs.90998
E74-like factor 1	(ELF1)	0.2891	Hs.154365
10. Others			
(known function)			
Proenkephalin	(PENK)	3.7120	Hs.93557
Translation factor suil homolog	(GC20)	3.6522	Hs.21756
Phosphodiesterase 6D	(PDE6D)	2.9083	Hs.48291
Glycosyltransferase AD-017	(AD-017)	2.4956	Hs.283737
Proline and glutamic acid rich nuclear protein	(PELP1)	2.4340	Hs.274149
Down syndrome critical region gene 3	(DSCR3)	2.3365	Hs.26146
N-methylpurine-DNA glycosylase	(MPG)	2.3330	Hs.79396
Keratocan	(KERA)	2.2918	Hs.125750
Dual specificity phosphatase 5	(DUSP5)	2.1058	Hs.2128
Cysteine and glycine-rich protein 1	(CSRPI)	0.4937	Hs.108080
Nuclear RNA helicase, DECD variant of DEAD box family	(DDXL)	0.4871	Hs.311609
Pituitary tumor-transforming 1 interacting protein	(PTTG1IP)	0.4474	Hs.111126
LIM domain-containing preferred translocation partner in lipoma	(LPP)	0.4460	Hs.180398
Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein	(YWHAB)	0.4391	Hs.279920
RNA binding motif, single stranded interacting protein 2	(RBMS2)	0.4299	Hs.20938
Protein phosphatase 4, regulatory subunit 1	(PPP4R1)	0.3920	Hs.3382
Ras-GTPase-activating protein SH3-domain-binding protein	(G3BP)	0.3403	Hs.220689
Parathyromosin	(PTMS)	0.3099	Hs.171814
Gene with multiple splice variants near HD locus on 4p16.3	(RES4-22)	0.2544	Hs.325987
Serine (or cysteine) proteinase inhibitor, clade E, member 1	(SERPINE1)	0.2132	Hs.82085
Thyroid hormone receptor interactor 10	(TRIP10)	0.1819	Hs.73999
Splicing factor 3b, subunit 2, 145kD	(SF3B2)	0.0446	Hs.75916
(unknown proteins and ESTs)			
Hypothetical protein FLJ22625	(FLJ22625)	4.1778	Hs.106534
KIAA0076 gene product	(KIAA0076)	3.2880	Hs.51039
FK506 binding protein 1A (12kD)	(FKBP1A)	3.0888	Hs.179661
FLJ21897 fis		2.9716	Hs.173108

Table I. (continued)

Name of gene	Symbol	Ratio	Unigene ID
Hypothetical protein FLJ22638		2.7472	Hs.183232
Hypothetical protein MGC4251	(MGC4251)	2.5765	Hs.74266
Homo sapiens cDNA FLJ10151 fis		2.5594	Hs.55424
Protein expressed in thyroid	(YF13H12)	2.3788	Hs.7486
ESTs		2.3181	Hs.104558
Hypothetical protein FLJ20186	(FLJ20186)	2.3094	Hs.65021
Hypothetical protein A-211C6.1		2.3079	Hs.28607
FLJ11658 fis		2.2645	Hs.22660
Hypothetical protein MGC13033	(MGC13033)	2.2040	Hs.26118
Hypothetical protein FLJ12886	(FLJ12886)	2.1189	Hs.10116
KIAA0202 protein	(KIAA0202)	0.4790	Hs.80712
Homo sapiens far upstream element (FUSE) binding protein 1	(FUBP)	0.3007	Hs.118962
KIAA0332 protein	(KIAA0332)	0.2290	Hs.7976

t test showed that there was no significant difference between Cy5/Cy3 ratios of good spots in two repeated experiments (Fig 2D), indicating the reproducibility of the differential expression results.

Functional analysis of the genes affected by red light The 111 genes affected by red light were classified into 10 categories according to their functions (Table I).

Category 1: Genes related to cell proliferation The first category consists of nine genes related to cell proliferation; six of them were upregulated. Eight of the genes play stimulatory roles in cell proliferation. For example, mitogen-activated protein kinase 11 (MAPK11) is an isoform of the p38 MAPK, and it is known that the p38 MAPK signaling pathway is involved in fibroblast growth factor 2 induced proliferation (Maher, 2002). Breakpoint cluster region (BCR) is a GTPase-activating protein for RAC1 and CDC42 that promotes the exchange of RAC- or CDC42-bound GDP by GTP. Active RAC1 and CDC42 can suppress p21, leading to the upregulation of BCR gene, which can enhance cell growth (Bao *et al*, 2002). Platelet-derived growth factor C (PDGF-C) is a member of the PDGF/vascular endothelial growth factor family and its upregulation can induce mitogenic activity on several mesenchymal cell types (Gilbertson *et al*, 2001). Serum response factor contributes to mitogen-stimulated transcriptional induction of many immediate-early genes during the G₀-G₁ cell cycle transition and is also essential for cell cycle progression (Schratt *et al*, 2001). The downregulated gene cullin 1 is an inhibitory regulator of the cell cycle. Cullin 1 is required for developmentally programmed transitions from the G₁ phase to the G₀ phase of the cell cycle or the apoptotic pathway, the mutation of which leads to the acceleration of G₁ to S phase progression (Kipreos *et al*, 1996). The downregulation of the gene may enhance cell growth.

Category 2: Antioxidant-related genes The second category includes two genes and both were upregulated. Selenoprotein W is a glutathione-dependent antioxidant, overexpression of which markedly reduced the sensitivity of cells to H₂O₂ cytotoxicity (Jeong *et al*, 2002). The intracellular peroxide concentration in cells overexpressing selenoprotein W was lower than that of the parental cells in the absence or presence of extracellular H₂O₂. The resistance to oxidative stress conferred by selenoprotein W was dependent on glutathione (Jeong *et al*, 2002). ATX1 antioxidant protein 1 is a member of the metallochaperone family that escorts copper to distinct intracellular targets. When overproduced, ATX1 substitutes for superoxide dismutase 1 in preventing oxidative damage by a metallochaperone mechanism and/or by direct consumption of superoxide (Lin and Culotta, 1995; Portnoy *et al*, 1999).

Category 3: Genes related to apoptosis or stress response The third category consists of five apoptosis- or stress-related genes. Three of

them were downregulated following red light irradiation, including heat shock 70 kDa protein 1 A, stress-induced phosphoprotein 1, and caspase 6. The downregulation of these genes may exert the effect of antiapoptosis. JAK binding protein, also known as STAT-induced STAT inhibitor 1 (SSI-1) or suppressor of cytokine signaling 1 (SOCS-1), is a negative feedback regulator of Janus kinase STAT signaling. The expression of the gene is increased upon irradiation. It was reported that the murine embryonic fibroblasts lacking the JAK binding protein gene are more sensitive than their littermate controls to tumor necrosis factor α induced cell death (Morita *et al*, 2000). The upregulation of JAK binding protein may also increase the survivability of the cells.

Category 4: Metabolism-related genes Twenty genes that mediate metabolism are grouped in the fourth category and can be further categorized into four subcategories, including genes related to protein and amino acid metabolism, sugar metabolism, lipid metabolism, and respiratory chain and energy metabolism.

Of the 12 genes related to protein and amino acid metabolism, seven were upregulated and five were downregulated. These genes are involved in the synthesis, procession, and turnover of peptides or amino acids. In the sugar metabolism subcategory, all of the genes were downregulated. They are enolase 3 and aldolase, a fructose-bisphosphate, both belonging to the glycolytic pathway (Horecker *et al*, 1972; Pancholi, 2001). In the lipid metabolism subcategory, both apolipoprotein C-III, an effective inhibitor of very low density lipoprotein triglyceride hydrolysis, and lysophospholipase II, a mediator of phospholipid degradation, were upregulated (Yokoyama *et al*, 1995; Jong *et al*, 2001). Apolipoprotein AI was downregulated; it is a member of the lipid-binding high-density lipoproteins (HDL) and participates in the transport of HDL cholesterol and other lipids in the plasma (Ladas and Karathanasis, 1991).

All three genes in the subcategory related to energy metabolism and respiratory chain were upregulated. NADH dehydrogenase (ubiquinone) 1 β subcomplex, 2 (8 kDa, AGGG) is one of the peptides of mitochondria respiratory complex I that transfer electrons from NADH to the respiratory chain (Weiss *et al*, 1991). ATP synthase, H⁺ transporting, mitochondrial F₀ complex, subunit d (ATP5H) belongs to the respiratory complex V (F₁F₀-ATPase assembly), which catalyzes ATP synthesis (Walker and Collinson, 1994). Electron-transfer-flavoprotein β polypeptide (ETF β) is a subunit of ETF that serves as a specific electron acceptor for several dehydrogenases, including acyl-CoA dehydrogenases that function in fatty acid β oxidation (Thorpe and Kim, 1995). ETF is reoxidized by electron transfer flavoprotein-ubiquinone oxidoreductase that contains FAD and a 4Fe4S cluster and transfers electrons to ubiquinone in the main respiratory chain (Ruzicka and Beinert, 1977). It has been proposed that mitochondrial stimulation by low-intensity laser is due to the generation of an extra electrochemical potential and an increase in ATP synthesis within mitochondria upon laser irradiation (Passarella *et al*,

1984; Wilden and Karthein, 1998). Our result suggests that the increase in ATP synthesis may be caused by upregulation of the expression of certain enzymes of the respiratory chain within mitochondria. The upregulation of ETF and the downregulation of enolase 3 and aldolase A may allow fibroblasts to use fatty acids more readily as the source of ATP synthesis.

Category 5: Genes related to ion channels, transport proteins, and membrane potential There are four upregulated genes in this category. ATP-binding cassette, subfamily A gene, is a member of the ATP-binding cassette A (ABCA1) transporter superfamily. The gene encodes a membrane protein that facilitates the cellular efflux of cholesterol and phospholipids. Mutations in ABCA1 lead to familial high-density lipoprotein deficiency and Tangier disease (Brooks-Wilson *et al*, 1999; Santamarina-Fojo *et al*, 2000). Upregulation of the gene may prevent the cells from over-accumulation of cholesterol and phospholipids. The other three genes are potassium voltage-gated channel, subfamily G member 1; potassium inwardly rectifying channel, subfamily J member 13; and α polypeptide of voltage-gated sodium channel, type IV. The gene encoding chloride intracellular channel 4 was downregulated. The change in the expression of these ion channel related genes may be required for cell proliferation, because cell proliferation must – at some time point – lead to an increase in cell volume. Activation of K^+ channels and a change in membrane potential have been observed in a wide variety of cells upon exposure to diverse mitogenic factors (Lang *et al*, 2000).

Category 6: Genes related to cytoskeleton and cell-cell interactions There are 17 genes in this category, which can be further divided into three subcategories, including cytoskeleton and cell motility, extracellular matrix, and migration, aggregation, and adhesion. Most genes in the category are functional and/or structural constituents of cell proliferation upon mitogenic stimulation.

Category 7: Genes related to DNA synthesis and repair All three genes in this category are upregulated. Nudix (nucleoside diphosphate linked moiety X) type motif 1 (NUDT1) is an antimutagenic gene, which encodes an enzyme that can hydrolyze 8-oxo-dGTP to 8-oxo-dGMP. NUDT1 (Sakumi *et al*, 1993), thereby preventing the misincorporation of 8-oxo-dGTP produced by active oxygen species normally formed during cellular metabolic processes. Adenine phosphoribosyltransferase (APRT) is involved in the formation of cAMP by catalyzing a salvage reaction. It is also a DNA repairing enzyme that participates in the rescue of cells from the toxic and carcinogenic effects of alkylating agents by hydrolysis of alkylated DNA, releasing 3-methyladenine, 3-methylguanine, 7-methylguanine, and 7-methyladenine (Samson *et al*, 1991).

Categories 8–10: Genes related to other functions Other categories of genes are affected upon red light irradiation, including genes related to transcriptional factors (category 8), immune/inflammation and cytokines (category 9), and genes with other known and unknown functions (category 10). The roles these genes play in fibroblasts irradiated by low-intensity red light are not clear.

Molecular mechanisms of the effects of red light irradiation on fibroblasts The gene expression profiles of human fibroblasts irradiated by low-intensity red light show that the irradiation can affect the expression of many genes that belong to different function categories. Among the 10 gene categories, seven of them are directly or indirectly involved in cell proliferation. Their participation in irradiation-induced fibroblast proliferation is illustrated in Fig 3. Irradiation of low-intensity red light stimulates cell growth directly through regulation of the expression of genes related to cell proliferation and indirectly through regulation of the expression of genes related to cell migration and remodeling, DNA synthesis and repair, ion channel and membrane potential, and cell metabolism. Irradiation by red light also enhances cell proliferation by suppression of cell apoptosis.

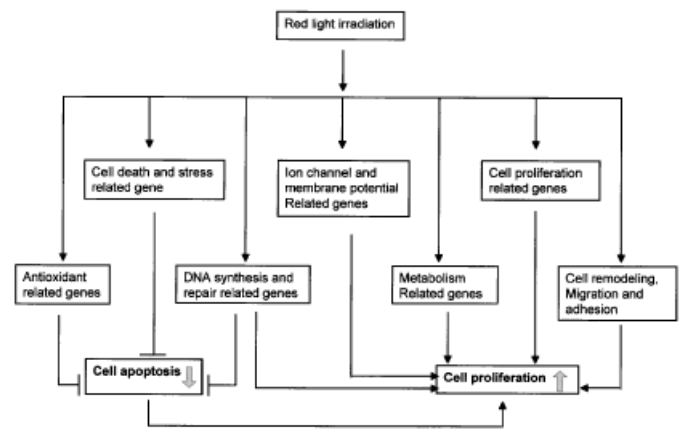


Figure 3. The involvement of various functional categories of differentially expressed genes in irradiation-induced fibroblast proliferation.

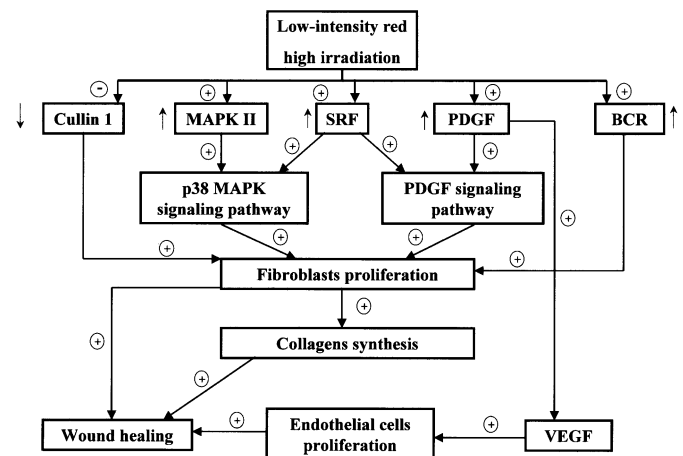


Figure 4. The roles of various differentially expressed genes in the signaling pathways that accelerate the proliferation of fibroblast and endothelial cells and eventually lead to wound healing. SRF, serum response factor, VEGF, vascular endothelial growth factor.

Cell proliferation is a very important factor in wound healing. It has been reported that the irradiation by low-intensity laser or red light accelerated the healing of skin wounds in animal models and in human by increasing the proliferation of cellular components for wound healing, promoting the synthesis of collagen content, and improving microcirculation in wounded areas (Reddy *et al*, 1998; Schindl *et al*, 2000). The fibroblast is one of the important cellular components of wound healing. Our results suggest that several genes related to cell proliferation might play major roles in fibroblast proliferation stimulated by red light irradiation. Some of these genes participate in either or both of the p38 MAPK signaling pathway and the PDGF signaling pathway. Therefore, both pathways might be involved in the proliferation of fibroblasts induced by red light irradiation. The expression of PDGF upon red light stimulation has been previously reported (Yu *et al*, 1994b). Because PDGF can increase the transcription and secretion of vascular endothelial growth factor by β -PDGFR-expressing endothelial cells (Wang *et al*, 1999), the upregulation of PDGF may not only play a role in the proliferation of fibroblasts but also take part in the stimulation of the growth of endothelial cells. The proliferation of endothelial cells can be regarded as a basis for the improvement of microcirculation by red light irradiation. Figure 4 shows the participation of various differentially expressed genes in the signaling pathways that might accelerate the proliferation of fibroblast and endothelial cells, eventually leading to wound healing.

In addition to the potential effects on wound healing, it has been reported that red light irradiation enhances the ability of the antioxidant system (Iakymenko and Sydoryk, 2001). The observation of an increase in the expression of two antioxidant genes after red light irradiation, selenoprotein W and ATX1 antioxidant protein 1, may provide a molecular insight into the increase in antioxidant ability. Previous studies have shown that consecutive, long-term exposure to red light increased collagen production (Conlan *et al*, 1996). Based on the expression profiling results, although the expression of collagen genes was not significantly upregulated, possibly due to the limited dose and duration of irradiation, several genes related to apoptosis were downregulated and some DNA repair genes were upregulated. It appears that red light irradiation might exert certain effects on genes related to cell growth, collagen production, microcirculation, and the ability for antiapoptosis, DNA repair, and antioxidation. Although some of these changes may be involved in reducing the progress of skin aging, additional experiments are needed to investigate the effects of red light on skin aging.

In summary, red light irradiation offers moderate stimulation for proliferation of HS27 fibroblasts under certain conditions. The cells responded to the irradiation dose as a nonlinear curve for low-intensity red light at 628 nm. The optimal dose to stimulate HS27 cell proliferation is 0.88 J per cm². Gene expression profiling of HS27 fibroblasts upon red light irradiation revealed that the expression of 111 genes was affected by red light and these genes could be grouped into 10 distinct categories based on their known functions, including proliferation, antioxidant, metabolism, ion channel and membrane potential, cytoskeleton and extracellular matrix proteins, DNA synthesis and repair, transcription factors, and immune/inflammation and cytokines. Most genes in seven of the 10 categories either directly or indirectly participate in various biologic processes related to cell proliferation. Two signaling pathways, the p38 MAPK signaling pathway and the PDGF signaling pathway, were identified to play important roles in mediating the effects of red light irradiation on the proliferation of HS27 fibroblasts. In addition to its effects on cell proliferation, red light irradiation may also regulate the expression of genes in relevant cells that are related to microcirculation, antiapoptosis, antioxidation, and DNA repair.

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