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► **To cite this version:**

Céline Borlon, Florence Debacq-Chainiaux, Christina Hinrichs, Karin Scharffetter-Kochanek, Olivier Toussaint, et al.. The gene expression profile of psoralen plus UVA-induced premature senescence in skin fibroblasts resembles a combined DNA-damage and stress-induced cellular senescence response phenotype. *Experimental Gerontology*, 2007, 42 (9), pp.911. 10.1016/j.exger.2007.04.009 . hal-00499013

**HAL Id: hal-00499013**

**<https://hal.science/hal-00499013>**

Submitted on 9 Jul 2010

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## Accepted Manuscript

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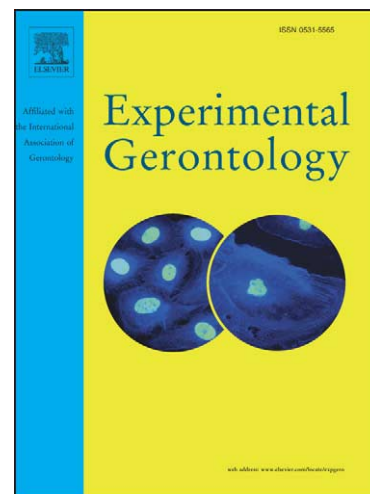
PII: S0531-5565(07)00089-7  
DOI: [10.1016/j.exger.2007.04.009](https://doi.org/10.1016/j.exger.2007.04.009)  
Reference: EXG 8336

To appear in: *Experimental Gerontology*

Received Date: 22 December 2006  
Revised Date: 19 April 2007  
Accepted Date: 24 April 2007

Please cite this article as: Borlon, C., Debacq-Chainiaux, F., Hinrichs, C., Scharffetter-Kochanek, K., Toussaint, O., Wlaschek, M., The gene expression profile of psoralen plus UVA-induced premature senescence in skin fibroblasts resembles a combined DNA-damage and stress-induced cellular senescence response phenotype, *Experimental Gerontology* (2007), doi: [10.1016/j.exger.2007.04.009](https://doi.org/10.1016/j.exger.2007.04.009)

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1 **Title**

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4 phenotype  
5  
6  
7

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30 **Running title :**

31 Gene profiling of PUVA-induced premature senescence  
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33  
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37 **Key words**

38 psoralen, ultraviolet A irradiation, cellular senescence, fibroblasts, gene expression, stress-  
39 induced premature senescence.  
40  
41

1 **Abstract**

2 After a finite number of population doublings, normal human cells undergo replicative  
3 senescence accompanied by growth arrest. We previously described a model of stress-induced  
4 premature senescence by treatment of dermal fibroblasts with psoralen plus UVA, a common  
5 photodermatological therapy. Psoralen photoactivation has long been used as a therapy for  
6 hyperproliferative skin disorders. The repetitive therapeutical treatment is accompanied by  
7 premature aging of the skin. Treatment of fibroblasts *in vitro* with 8-methoxypsoralen (8-MOP)  
8 and subsequent ultraviolet A (UVA) irradiation results in growth arrest with morphological and  
9 functional changes reminiscent of replicative senescence. For gene expression profiling in two  
10 strains of human skin fibroblasts after PUVA treatment, we used a low density DNA array  
11 representing 240 genes involved in senescence and stress response. Twenty-nine genes were  
12 differentially expressed after PUVA treatment in the two strains of human skin fibroblasts. These  
13 genes are involved in growth arrest, stress response, modification of the extracellular matrix and  
14 senescence. This study contributes further to the elucidation of the PUVA model and its  
15 validation as a useful stress-induced premature senescence model aiming to characterize the  
16 premature senescence of fibroblasts and to identify biomarkers that could be applied *in vivo*.

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18

## 1 1. Introduction

2 There is now increasing evidence that cellular senescence *in vitro* reflects – at least in part –  
3 cellular aging *in vivo* (Patil et al., 2005). Recently, the development of different premature  
4 senescence inducing models has extended our knowledge of basic molecular mechanisms.  
5 Extrinsic as well as intrinsic cellular senescence mechanisms overlap *in vivo*. The underlying  
6 molecular mechanisms can be analyzed in detail by inducing premature senescence in dermal  
7 fibroblasts *in vitro*. In dermatological phototherapy, the therapeutic properties of psoralens have  
8 been used for long time. The antiproliferative effects of photosensitized psoralens are related to  
9 the ability of their tricyclic aromatic structure to absorb ultraviolet radiation and to generate  
10 DNA-damage. PUVA-therapy with 8-methoxypsoralen and long-wave ultraviolet-A (320-400  
11 nm) irradiation has been used in the treatment of inflammatory and neoplastic skin disorders like  
12 psoriasis and T-cell lymphoma. Interestingly, premature aging of the skin has been observed in  
13 these patients (Sator et al., 2002). Application of PUVA treatment to dermal fibroblasts induced a  
14 long term switch of mitotically active fibroblasts to post-mitotic fibroblasts mimicking several  
15 features of replicative senescence (Herrmann et al., 1998; Ma et al., 2003; Wlaschek et al., 2003),  
16 including the activation of the senescence associated  $\beta$ -galactosidase (SA- $\beta$ -gal), a biomarker of  
17 fibroblast senescence *in vivo* and *in vitro* (Dimri et al., 1995). Reactive oxygen species are  
18 causally involved in the PUVA-induced permanent growth arrest since superoxide anion radicals  
19 and hydrogen peroxide were identified as causal intermediates in the downstream signaling  
20 pathway leading to the response of fibroblasts to PUVA treatment (Herrmann et al., 1998). A  
21 permanent increase in the synthesis of the matrix-metalloproteases MMP1 and MMP3 (Herrmann  
22 et al., 1998) has been observed. Using subtractive hybridization, the genes for the cyclin-  
23 dependent kinase inhibitor p21<sup>Waf-1</sup>, ferritin-H and tissue inhibitor of metalloproteinase-3 (TIMP-  
24 3) have been shown to be up-regulated and the genes for apolipoprotein D, laminin B1 and  
25 pleiotrophin have been shown to be down-regulated (Wlaschek et al., 2000a; Wlaschek et al.,  
26 2000b).

27 In order to compare in more detail the functional phenotype of cellular senescence with the  
28 phenotype obtained following PUVA treatment and other stress-induced premature senescence  
29 models, we performed a gene expression and time kinetic study with different fibroblast strains  
30 using a low density DNA array representing 240 genes involved in cellular senescence and stress  
31 response of human diploid fibroblasts. A selected data set was verified using quantitative RT-  
32 PCR. We report that the gene expression profile found in fibroblasts submitted to PUVA  
33 treatment is comparable to other types of stress induced premature senescence of fibroblasts. We  
34 detected genes involved in growth arrest, stress response, modification of extracellular matrix and  
35 signal transduction. The reliability of this technology has been fully demonstrated previously (de  
36 Longueville et al., 2002; de Magalhaes et al., 2004; Debacq-Chainiaux et al., 2005; Pascal et al.,  
37 2005). This technology was already used successfully in different models of stress-induced  
38 premature senescence like H<sub>2</sub>O<sub>2</sub>-induced premature senescence of skin HDFs with and without  
39 ectopic expression of telomerase (de Magalhaes et al., 2004; Zdanov et al., 2006) and UVB-  
40 induced premature senescence of skin HDFs (Debacq-Chainiaux et al., 2005).

41

## 1 2. Materials and methods

### 2 2.1. Cell Culture and PUVA treatment

3 AG04431 fetal skin HDFs (Corriell Institute For Medical Research, USA) were grown in DMEM  
4 (Dulbecco's Modified Eagle's Medium) (GIBCO-BRL, Eggenstein, Germany) supplemented  
5 with 10% FCS (Fetal Calf Serum) (Biochrom, Berlin, Germany), 2mM L-glutamine, 100 µg/ml  
6 streptomycin (Biochrom, Berlin, Germany). Cells were used at 50 – 60 % of *in vitro* life span.  
7 FF95 fibroblast cultures were established by outgrowth from foreskin of a healthy donor of the  
8 age of one year according to the method described earlier (Fleischmajer et al., 1981). The cells  
9 were maintained in DMEM supplemented with 10% FCS, 2mM L-glutamine, 50 µg/ml  
10 streptomycin and penicillin (400 U/ml). Cells were used at cumulative population doublings  
11 (CPD) between 10 and 20. Fibroblasts were detached at various days following treatment and cell  
12 numbers were determined as described (Bayreuther et al., 1988). Cells were cultured in a  
13 humidified atmosphere with 5% CO<sub>2</sub> and 95% air at 37°C. Following PUVA-induced growth  
14 arrest cells were fed weekly by changing half of the medium.

15 Crystalline 8-methoxypsoralen (8-MOP) (Sigma, Deisenhofen FRG) was dissolved at 1 mg/ml in  
16 DMSO and stored in the dark. A final concentration of 50 ng/ml was prepared freshly in DMEM  
17 or phosphate buffered saline pH 7.4 (10 mM phosphate, 0,9 % NaCl) (PBS) directly prior to use.  
18 For PUVA treatment, fibroblasts were seeded at a cell density of  $3.5 \cdot 10^3 / \text{cm}^2$  and incubated for  
19 24 h. Subsequently, cells were pre-incubated for 16h with 50 ng/ml of 8-MOP in DMEM. UVA-  
20 irradiation was performed on cells incubated with PBS +/- 8-MOP. The cells were irradiated with  
21 90 kJ/m<sup>2</sup> using a high intensity UVA source (UVASUN 3000 equipped with the UVASUN safety  
22 filters) emitting wavelengths in the 340-450 nm range (Mutzhas et al., 1981) as published  
23 (Herrmann et al., 1998). Fluences were determined with a UVA-ultraviolet meter (Dr. Hönle,  
24 Martinsried). The emission spectrum of the UV source was checked on a regular basis with an  
25 Optronics OL-754 radiospectrometer (Optronics, Orlando, FL).

### 26 2.2. Cytotoxicity assay

27 The viability of the fibroblasts after PUVA treatment was measured 24 hours after UV-irradiation  
28 using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) by  
29 quantifying living metabolically active cells. Mitochondrial dehydrogenases metabolize MTT to  
30 purple formazan dye (Green et al 1984). Cytotoxicity was calculated as percentage of formazan  
31 formation in cells treated with the PUVA regimen compared to mock-treated cells.

32 We performed the viability assay for both cell strains. Similar to other fibroblast strains  
33 (Herrmann et al 1998), at 24 h after UV-irradiation of the fibroblasts treated with the 8-MOP, the  
34 viability was at least 80% compared to mock treated fibroblasts of the same strain.

### 35 2.3. Low density DNA-Array

#### 36 *Microarray design and hybridization*

37 The DualChip™ human aging is a low-density DNA array developed in collaboration with  
38 Eppendorf (Germany). This array, representing a range of 240 genes involved in senescence and  
39 stress response of HDFs, contains two arrays per glass slide (a control and a test) with three  
40 subarrays per array. The sequences of the DNA covalently linked to the glass slide were carefully  
41 chosen by sequence comparison. Checks were made to ensure that no cross-hybridization takes  
42 place. To evaluate the reliability of the experimental data, several positive and negative  
43 hybridization and detection controls are included on the microarray. For normalization, internal  
44 standard controls and housekeeping genes were arrayed on the slides.

45 Total RNA was extracted from fibroblasts at 24 h, 72 h, 1 week and 4 weeks after PUVA  
46 treatment using TRIfast (Peqlab, Germany). Quality control of total RNA was controlled on  
47 agarose gels. The amount of RNA was determined spectrophotometrically at 260 nm. Total RNA  
48 (15 µg) was reverse transcribed using SuperScript II Reverse Transcriptase (Invitrogen, UK).  
49 Three independent experiments were performed. Hybridization on DualChip human aging was

1 carried out as described by the manufacturer. Detection was performed using a Cy3-conjugated  
2 IgG anti-biotin (Jackson Immuno Research Laboratories, USA).

### 3 *Imaging, data normalization and statistical analysis*

4 Fluorescence of the hybridized arrays was scanned (Packard ScanArray, PerkinElmer, USA) at a  
5 resolution of 10  $\mu\text{m}$ . To maximize the dynamic range of detection, the same arrays were scanned  
6 at three photomultiplier gains (50, 70, 100). The scanned 16-bit images were imported into the  
7 ImaGene 4.1 software (BioDiscovery, USA). The fluorescence intensity of each DNA spot  
8 (average intensity of each pixel present within the spot) was calculated using local mean  
9 background subtraction. A signal was accepted when the average intensity after background  
10 subtraction was at least 2.5-fold higher than its local background. The three intensity values of  
11 the triplicate DNA spots were averaged and used to calculate the intensity ratio between the  
12 reference and the test samples.

13 The data were normalized in two steps. First, the values were corrected using a factor calculated  
14 from the intensity ratios of the 6 internal standards in the reference and test samples. These  
15 internal standards were located at two different locations on the array allowing to consider the  
16 local background and array homogeneity in the normalization process. The internal standard  
17 control does not take into account the purity and quality of the mRNA. Thus, a second step of  
18 normalization was performed based on calculating the average intensity for a set of eight  
19 housekeeping genes. This set of multiple housekeeping genes, with different expression levels,  
20 was preferred to a single reference gene to avoid any bias due to possible variability of the  
21 expression level of a single housekeeping gene. Among these housekeeping genes, only genes for  
22 which the expression was not changed after a particular treatment were taken into account for the  
23 normalization. The variance of the normalized set of housekeeping genes (except those affected  
24 by the treatment) was used to generate an estimate of expected variance, leading to a predicted  
25 confidence interval to test the significance of the ratios obtained. Ratios outside the 95%  
26 confidence interval were determined to be significantly different (de Longueville et al., 2002; de  
27 Magalhaes et al., 2004; Debacq-Chainiaux et al., 2005; Pascal et al., 2005; Zdanov et al., 2006).

### 28 *2.4. Validation of relative gene expression by Real Time RT-PCR*

29 Total RNA (2  $\mu\text{g}$ ) was reverse transcribed using SuperScript II Reverse Transcriptase  
30 (Invitrogen, UK). Primers (Table 1) were designed using the Primer express 1.5 software  
31 (Applied Biosystems, The Netherlands). These specific primers correspond to the gene sequence  
32 present on the DualChip<sup>TM</sup> human aging (Eppendorf, Hamburg, Germany). Amplification  
33 reaction assays contained 1X SYBER Green PCR Mastermix (Applied Biosystems, The  
34 Netherlands), and primers at optimal concentration. PCR reactions without cDNA were  
35 performed as template-free negative controls. All PCR reactions were made in duplicates with a  
36 hot start at 95 °C for 5 minutes, followed by 40 cycles at 95 °C for 15 seconds and 65°C for 1  
37 minute using the 7000 SDS thermal cycler (Applied Biosystems, The Netherlands). Melting  
38 curves were generated after amplification.



### 3. Results

PUVA treatment results in premature aging of the skin *in vivo* with morphological and functional changes mimicking replicative senescence of fibroblasts *in vitro*. Distinct features of cellular senescence are observed following PUVA treatment of fibroblasts *in vitro*. These features include long-term growth arrest, senescent morphology with increased cytoplasm surface, activity of senescence associated- $\beta$  galactosidase, and the increased expression of interstitial collagenase (MMP-1) (Herrmann et al., 1998). In order to find new markers for this stress-induced senescence and to compare the expression profile with other stress-induced premature senescence models to identify common pathways, we used a senescence specific low-density DNA array to estimate the relative mRNA levels of 240 genes involved in senescence and stress response in the two skin fibroblasts strains AG04431 and FF95, submitted to PUVA treatment at the same CPD, as detailed in Materials and Methods. Mock-treated control cells were submitted to all changes of media without 8-MOP pre-incubation and UVA irradiation. The list of the 240 genes represented on this array is available (Debacq-Chainiaux et al., 2005). Total RNA was extracted at 24 h, 72 h, 1 week and 4 weeks after PUVA treatment. 15  $\mu$ g RNA were retrotranscribed as described in the Materials and Methods. All cDNA samples were hybridised in triplicates.

We obtained reproducible hybridization patterns on the DualChip™ human aging for both strains of fibroblasts. Scanning, data mining, normalization and statistical treatment followed previously established procedures. Around 100 genes were found in the triplicates to be differentially expressed in PUVA-induced premature senescence. This large number of variation is not very surprising since all the genes represented on the array are senescence and stress-related. The expression changes of some genes were cell type specific while for others they occurred in a time dependent manner.

Fifty genes showed increased and 44 genes showed decreased transcript levels in FF95 fibroblasts after PUVA treatment. Forty-nine and 14 genes revealed increased and decreased transcript levels in AG04431 fibroblasts, respectively, after PUVA treatment (Figure 1). These genes are listed in table 2 for FF95 and table 3 for AG04431. A consistent proportion of genes were similarly differentially expressed in both strains of fibroblasts with 23 genes with increased transcript level and 6 genes with decreased transcript level (Table 4).

Most of these genes could be grouped according to their potential involvement in the PUVA-induced phenotype. Here, we present a more detailed analysis for all these commonly differentially expressed genes that can have a potential involvement in the SIPS phenotype reflecting – at least in part – cellular senescence.

#### Growth and cell-cycle regulated genes

The cyclin-dependent kinase inhibitor  $p21^{WAF-1}$ , involved in cell cycle control by regulating cell cycle arrest at the G1/S transition (Sherr and Roberts, 1999), was increased as previously described in replicative senescence. The data on  $p21^{WAF-1}$  expression confirm the earlier observation that  $p21^{WAF-1}$  is induced at the RNA and protein level following PUVA treatment of fibroblasts (Ma et al., 2003; Wlaschek et al., 2000a). The *proliferating cell nuclear antigen* (PCNA) is a gene involved in DNA repair and DNA replication (Xu and Morris, 1999).

*Cyclin D1* (*CCND1*) was up-regulated in a similar fashion to replicative senescence (Fukami et al., 1995). The up-regulated *Cyclin G1* (*CCNG*) is a damage inducible gene (Bates et al., 1996), and plays a role in modulating apoptosis induced by different stimuli (Okamoto and Prives, 1999). The *Connective Tissue Growth Factor* (*CTGF*) was already shown to be down-regulated after UV irradiation. The mechanisms of this down regulation are not yet known (Rittie and Fisher, 2002).

*Metallothionein 2A* (*MT2A*) is downregulated and may be involved in the regulation of cell proliferation and apoptosis. Overexpression of *MT2A* can promote tumorigenesis (Simpkins, 2000) (Jin et al., 2001).



1 Genes related to stress response

2 Different genes involved in stress response were upregulated. These include the *DNA damage*  
 3 *inducible transcript 3 (GADD153)* (Park et al., 1992), the *heat shock 90kD protein 1 alpha*  
 4 (*HSP90- $\alpha$* ) (Humphreys et al., 1999) and *heme oxygenase (HMOX)*. *HMOX* has been shown to  
 5 be up-regulated in stress response (Poss and Tonegawa, 1997). Additionally, different antioxidant  
 6 enzymes were upregulated including the *anti oxidant protein 2 (AOP2)*, also called  
 7 *peroxiredoxin VI (PRX VI)*, *manganese superoxide dismutase (SOD2)*, *glutathione peroxidase*  
 8 (*GPX*) (Fisher et al., 1999) and *glutathione S-transferase theta 1 (GSTT1)*. *GSTT1* was shown to  
 9 influence tumorigenesis through the transport of oxidized products generated by UV (Strange et  
 10 al., 1998).

11 *Annexin 1 (ANXI)* is a calcium-regulated membrane-binding protein, able to block cell migration.  
 12 *ANXI* shows a co-localization with the stress fibres (Babiychuk et al., 2002). The up-regulation  
 13 of *ANXI* could be linked to the increase of the number of stress fibres previously described in  
 14 premature senescence in, for instance,  $H_2O_2$ -induced premature senescence of human fibroblasts  
 15 (Chen et al., 2000).

16  
 17 Possible effectors in senescence-related genes

18 *Transforming Growth Factor Beta 1 (TGF $\beta$ -1)* has been previously shown to be over-expressed  
 19 after exposure of skin fibroblasts to UVB (Chainiaux et al., 2002; Debacq-Chainiaux et al., 2005)  
 20 and plays a causal role in the molecular mechanism underlying stress-induced premature  
 21 senescence.

22 *Cathepsin H (CTSH)* is associated with protein metabolism and linked to tumor progression  
 23 (Budihna et al., 1996; Waghray et al., 2002). We also detected  *$\beta$ 1-galactosidase (GLB1)*, one of  
 24 the senescence biomarkers (Dimri et al., 1995), which has been described as over-expressed after  
 25 PUVA treatment (Herrmann et al., 1998).

26 Some factors involved in oncogenesis are over-expressed such as *R-ras*, a member of the Ras  
 27 superfamily of small GTP-binding proteins, with a potential transforming activity and a possible  
 28 role in apoptotic growth control (Cox et al., 1994). *SHC transforming protein 1 (SHC)* was  
 29 described as a part of a signal transduction pathway that regulates stress apoptotic responses and  
 30 life span in mammals (Migliaccio et al., 1999).

31  
 32 Extracellular matrix-associated genes

33 We found over-expression of *metalloproteinase 14 (MMP14)*, and down-regulation of  
 34 *metalloproteinase 1 (MMP1)*, *collagen I-alpha (COL1A1)*, and *collagen III-alpha (COL3A1)*  
 35 (Rittie and Fisher, 2002).

36  
 37 Some genes could not be grouped into one of these categories. *Urokinase receptor (PLAUR)*,  
 38 involved in signal transduction was up-regulated (Borglum et al., 1992). *Keratin 19 (KRT19)* was  
 39 already shown to be over-expressed after stress, with its expression depending on the UV  
 40 wavelength in keratinocytes (Bernerd et al., 2001). *Ornithin decarboxylase 1 (ODC)* is up-  
 41 regulated after PUVA treatment. It was shown that UVB light induces overexpression of *ODC*,  
 42 an enzyme that plays a critical role in photocarcinogenesis (Jansen et al., 2001; Lee et al., 2003).  
 43 *ODC* activity can be considered as a marker of the mutagenic and carcinogenic effects of UV  
 44 light (Manzow et al., 2000). *BCLX* takes part in apoptosis regulation and plays an anti-apoptotic  
 45 role (Konopleva et al., 1999). It is up-regulated after PUVA treatment. *Histone 2b member B/S*  
 46 *consensus (HB2/S)* is upregulated and *histone 4 member M consensus (H4FM)* is downregulated  
 47 after PUVA. Because histones are the basic nuclear proteins responsible for the nucleosomal  
 48 structure of the chromosomal fibers in eukaryotes, they contribute to virtually all chromosomal  
 49 processes, such as gene regulation, chromosome condensation, recombination, and replication.

50  
 51 To verify the expression data generated with the DualChip Human Aging we used quantitative  
 52 real time RT-PCR of the selected genes *p21<sup>WAF-1</sup>*, *HMOX*, *CTGF*, *MT2A* and *SOD2* (Table 5)  
 53 known to play an important role in cellular senescence and stress response. A cut off of 1.3 was

1 fixed to select for high relevance. Differential expression observed with real time RT-PCR was  
2 higher than with the DNA microarray, because of the sensitivity of the technique. Nevertheless,  
3 comparable expression values with the DNA arrays and Real-Time RT-PCR were obtained.  
4

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#### 4. Discussion

It was previously shown that a single exposure of human dermal fibroblasts to combined 8-methoxypsoralen and UVA irradiation resulted in a cell response related to replicative senescence featuring growth arrest, expression of SA  $\beta$ -galactosidase (Herrmann et al., 1998), and up-regulation of MMP-1 and MMP3 (Herrmann et al., 1998), p21<sup>waf-1</sup>, TIMP-3 and ferritin H (Wlaschek et al., 2000a). To better characterize the cellular phenotype, we performed a gene expression study with two different skin fibroblast strains using a low-density DNA array representing 240 genes involved in senescence and stress response and remind only common variations to select more relevant results. Results were confirmed by Real Time RT-PCR.

All gene expression changes observed in this study can link the PUVA-induced changes to a state close to Stress-Induced Premature Senescence. In fact, the differential expression levels observed are similar to the ones observed in a study performed on human fibroblasts in which stress induced premature senescence was induced by UVB (Debacq-Chainiaux et al., 2005).

Growth arrest is an important feature of cellular senescence and stress induced premature senescence. Normal somatic cells enter a state of irreversibly arrested growth after a finite number of divisions (Campisi et al., 1996a; Cristofalo and Pignolo, 1993; Hayflick, 1965). It was already shown that fibroblasts submitted to psoralen photoactivation instantly switch from mitotically active to postmitotically cells even at low CPD (Herrmann et al., 1998). We can correlate this decrease of proliferative potential with the over-expression of p21<sup>waf-1</sup> and CCND1 in both strains of fibroblasts, and with the over-expression of p16<sup>INK4A</sup> in the AG04431 fibroblasts. In fact, recently we were able to identify p16<sup>INK4A</sup> as a robust biomarker for aging *in vivo* (Ressler et al., 2006). Similar results were found in SIPS induced by UVB in dermal fibroblasts (Debacq-Chainiaux et al., 2005).

The increased number of SA  $\beta$ -galactosidase positive cells is a biomarker for senescence *in vitro* and *in vivo* (Dimri et al., 1995) as well as for cells entered into SIPS (Dumont et al., 2000). Fibroblasts submitted to PUVA treatment are positive for SA  $\beta$ -galactosidase (Herrmann et al., 1998; Lee et al., 2006). Here, we confirm the overexpression at the mRNA level of  $\beta$ -galactosidase in both strains of fibroblasts after PUVA treatment.

It is known that oxidative DNA damage, due to reactive oxygen species, contributes to growth arrest in human diploid fibroblast cells (Chen et al., 1995). In PUVA-induced premature senescence we have shown that ROS are involved in the phenotype of premature senescence (Herrmann et al., 1998). In this study we found several antioxidant enzymes up-regulated such as AOP2, GPX, SOD2 and HMOX. AOP2 was detected to be up-regulated in other studies of stress induced premature senescence following UVB irradiation (Debacq-Chainiaux et al., 2005), or by *tert*-butylhydroperoxide (Dierick et al., 2002a; Dierick et al., 2002b). Its overexpression was shown to protect cells against phospholipid peroxidation-mediated membrane damage (Manevich et al., 2002), and cytotoxic stress (Dierick et al., 2003; Salmon et al., 2004). GPX and HMOX were already described to show overexpression in fibroblasts entered in SIPS following UVB irradiation (Debacq-Chainiaux et al., 2005).

Recently, it was shown that TGF- $\beta$ 1 plays a causal role in the senescence phenotype after UVB stress (Debacq-Chainiaux et al., 2005), as well as H<sub>2</sub>O<sub>2</sub> stress (Fripiat et al., 2001). Similarly, we found an upregulation in the mRNA levels of TGF- $\beta$ 1 which suggests the usefulness of future more detailed analyses of the role of this cytokine in the development of the senescence phenotype after PUVA treatment. Interestingly, the cellular stress response is not limited to the expression of immediate early genes within 24 hours but the response on mRNA levels occurs for some genes, like *SOD2*, after 1 week. From this data, it can be concluded that adaptive processes are involved. In fact, especially for genes included in stress response and in cell cycle arrest, we observed that the majority of these genes is up-regulated at early time points after stress, and this up-regulation is maintained for a long time, revealing a characteristic of the adaptive response after the PUVA stress.

1 PUVA induced premature senescence has been described as an *in vitro* model for stress-induced  
2 premature senescence. Some of the morphological and functional changes in the fibroblast  
3 phenotype following exposure to PUVA are described in detail (Ma et al., 2002). This study  
4 contributes to the further characterization of the PUVA model.

5 The differences in the transcript levels in both fibroblast strains may reflect the inter-individual  
6 variability as well as the fact that AG04431 fibroblasts are derived from fetal skin – known to  
7 have different expression patterns compared to juvenile and adult fibroblasts i.e. in wound  
8 healing – whereas FF95 fibroblasts were isolated from the foreskin of a one-year-old boy.  
9 Additionally, it has been noticed that fibroblasts of different body localization show – at least in  
10 part – different gene expression patterns.

11 In fact, some studies already demonstrated that replicative senescence in fibroblasts can be linked  
12 to a state that mimics the early remodeling phase of wound repair (Shelton et al., 1999; Yoon et  
13 al., 2004) with an up-regulation of inflammatory and immune responsive genes, and an  
14 upregulation of genes involved in cell cycle arrest. Some premature aging disorders have been  
15 linked directly to normal aging according to their gene expression profiles (Kyng et al., 2003).

16 Our data reveal further evidence that the cellular stress response involves the upregulation of  
17 genes specifically involved in the DNA-damage response in cellular senescence. Cellular  
18 senescence has been established recently as a DNA- and stress-damage response phenomenon  
19 (d'Adda di Fagagna et al., 2003; Di Micco et al., 2006; Hovest et al., 2006; von Zglinicki et al.,  
20 2005). This observation further substantiates the value of stress-induced cellular senescence  
21 models to delineate the underlying cellular and molecular mechanisms of regulation in cellular  
22 senescence. For the phototherapeutical treatment of patients with hyperproliferative skin  
23 disorders, our investigation will help in future to define adequate treatment schedules in respect  
24 to minimize unwanted side effects.

25 The changes at the mRNA level observed herein after PUVA treatment may better reflect the  
26 gene regulatory changes mediated by PUVA treatment during establishment of SIPS than the  
27 changes at the protein level. However, it is very often observed that an alteration in mRNA levels  
28 does not lead to a similar alteration in the protein abundance (Gygi et al., 1999). Studies at the  
29 protein level now could be performed for each of the PUVA-regulated genes identified herein.  
30 Such studies would confirm whether the changes observed at the mRNA level translate into  
31 changes at the protein expression level.  
32

### 33 **5. Acknowledgements**

34 This work was funded in part by the European Union through the CELLAGE-project (QLK6-CT-  
35 2001-00616), the individual grants SCHA411/8-2,-3,-4 by the german research foundation DFG  
36 to KSK. We acknowledge the Région Wallonne/FSE First-Europe projects "Arrayage" (C.  
37 Borlon) and "CosmUV", First-DEI project "Cosmet-X" and First-Spin-Off "Toxico-SIPS". We  
38 acknowledge the Région Wallonne "Réseaux II, Senegene" project, "Nanotoxico" project and  
39 "Walnut-20". We also thank the European Commission ('Geha' Project, LSHM-CT-2004-503270;  
40 'Link-Age' Project, LSHM-CT-2005-523866, Marie Curie-Transfer of Knowledge 'Matiss'  
41 Project, MTKI-CT-2006-042768). O. Toussaint and F. Debacq-Chainiaux are respectively  
42 Research Associate and Post-doctoral Researcher of the FNRS, Belgium.

43 We thank Dr. Joao-Pedro de Magalhaes for critically reading the manuscript.

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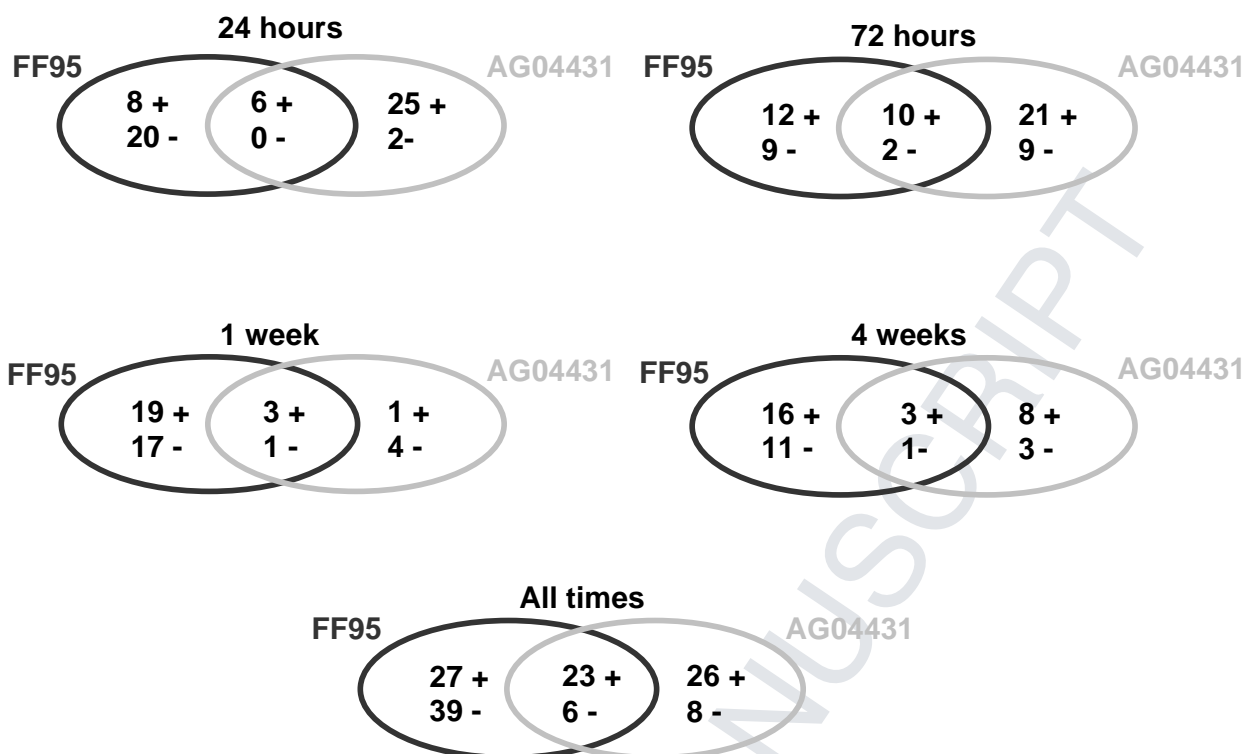


Figure 1: Sorting of the numbers of genes with differential expression after PUVA treatment (increased (+) or decreased (-) transcript abundance). Total RNA was collected at 24 h, 72 h, 1 week and 4 weeks after PUVA treatment.

Genes	Positions (bp)	Sequences
p21 <sup>WAF-1</sup>	495-515	5' - CTG GAG ACT CTC AGG GTC GAA - 3'
	599-617	5' - CCA GGA CTG CAG GCT TCC T - 3'
HMOX	799-820	5' - CCA GCA ACA AAG TGC AAG ATT C - 3'
	925-946	5' - CAC ATG GCA TAA AGC CCT ACA G - 3'
CTGF	242-258	5' - CAA GCT GCC CGG GAA AT-3'
	365-384	5' - GGA CCA GGC AGT TGG CTC TA -3'
MT2A	134-154	5' - GCA CTT CGT GCA AGA AAA GCT - 3'
	184-201	5' - GCA GCC TTG GGC ACA CTT - 3'
SOD2	632-653	5' - CAA ATT GCT GCT TGT CCA AAT C - 3'
	607-716	5' - CGT GCT CCC ACA CAT CAA TC - 3'
GAPDH	942-963	5' - ACC CAC TCC TCC ACC TTT GAC - 3'
	1033-1053	5' - GTC CAC CAC CCT GTT GTC GTA - 3'

Table 1: Primers used for quantitative Real Time RT-PCR

1 Table 2 (A) Genes overexpressed (control=1.00)

7. Name	Genbank	Gene	24 h	72 h	1 Week	4 Weeks
<b>8. Apoptosis / Inflammation</b>						
BCLX	NM_001191	BCLX	2.14	2.40	1.97	2.42
<b>Cell adhesion / Circulation</b>						
Thrombospondin	X14787	THBS1			2.53	2.02
Plasminogen activator inhibitor type 1	M14083	PAI1				1.79
Plasminogen activator inhibitor type 2	J02685	PAI2		2.42		
<b>Cell cycle</b>						
Cyclin D1	NM_053056	CCND1		2.06		2.73
Cyclin dependent kinase inhibitor 1A	U03106	p21	9.77	4.26	5.03	2.78
Cyclin G	U53328	CCNG			2.00	
Proliferating cell nuclear antigen	NM_002592	PCNA	1.84	2.15		
<b>Cell differentiation</b>						
Keratin17	X62571	KRT17	5.32			
Keratin19	NM_002276	KRT19		2.32		
Inhibitor of DNA binding 1	X77956	ID1	2.63			
<b>Cell signalling / receptor</b>						
Nuclear receptor subfamily 1	NM_021724	EAR1	3.39			
Urokinase receptor	NM_002659	PLAUR		1.71		
Early growth response 1	NM_001964	EGR1	1.62	2.54		
<b>Chromosomal processing</b>						
Histone 2b member B/S consensus	NM_080593	H2B/S	1.60			4.43
<b>Extracellular matrix / Cell structure</b>						
Matrix metalloproteinase 2	NM_004530	MMP2			2.07	1.73
Matrix metalloproteinase 3	NM_002422	MMP3		1.72	2.17	2.42
Matrix metalloproteinase 7	NM_002423	MMP7			2.76	
Matrix metalloproteinase 10	NM_002425	MMP10			3.39	
Matrix metalloproteinase 14	NM_004995	MMP14				2.97
Tissue inhibitor of metalloproteinase 2	NM_003255	TIMP2			1.67	
Osteonectin	NM_003118	Oste			1.86	2.30
Fibronectin	X02761	FN1		2.02		
Collagen III-alpha1	NM_000090	COL3A1			2.16	
Metalloprotease 1	AF061243	MP1		1.92		
Transgelin	M95787	SM22		2.98		
<b>Growth factors and cytokines</b>						
Insulin growth factor binding protein 2	M35410	IGFBP2				1.89
Insulin growth factor binding protein 5	M65062	IGFBP5			3.12	6.59
Connective tissue growth factor	U14750	CTGF		1.81		4.35
Protease serine 1	NM_002775	PRSS11			1.88	
Interleukin 1B	M15330	IL1B		1.78		
Transforming Growth Factor beta1	NM_000660	TGFB1	2.02			
Bone morphogenic protein 2	NM_001200	BMP2	2.92			
<b>Intermediate / Protein metabolism</b>						
Beta1 galactosidase	M34423	GLB1			1.79	2.05
Creatin kinase brain	M16364	CKB		1.53		2.05
Ornithin decarboxylase 1	NM_002539	ODC		3.21		
Cathepsin D	M11233	CTSD			1.98	2.10
Cathepsin H	NM_004390	CTSH				5.92
<b>Oncogenesis</b>						
SHC transforming protein1	U73377	SHC			1.98	
R-ras	NM_006270	RRAS			2.52	
<b>Stress response</b>						
Heme oxygenase	NM_002133	HMOX	2.88	2.60		2.13
Anti oxidant protein 2	NM_004905	AOP2		1.59	1.87	
Heat shock 90kD protein1 alpha	X15183	HSP90-alpha		2.07		
DNA damage inducible transcript 3	S40706	GADD153	4.32	2.35		
Superoxide dismutase	NM_000636	SOD2			17.74	1.96
Glutathione peroxidase	M21304	GPX	2.33	1.80	1.75	
Glutathione S-transferase theta 1	NM_000853	GSTT1			1.57	
Annexin I	NM_000700	ANX1		2.69		

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1  
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Table 2 (B) Genes down-regulated (control=1.00)

Name	Genbank	Genes	24 h	72 h	1 week	4 weeks
<b>Apoptosis / Inflammation</b>						
Prostaglandin endoperoxidase synthase 1	NM_000962	COX1	-2.44	-2.41		-3.10
Prostaglandin endoperoxidase synthase 2	NM_000963	COX2		-2.38		
<b>Cell adhesion / Circulation</b>						
Small inducible cytokine A5	NM_002985	RANTES			-1.48	
Plasminogen activator tissue	NM_000930	TPA			-2.17	
Urokinase	NM_002658	PLAU			-1.71	-2.71
<b>Cell cycle</b>						
Thymidine kinase	NM_003258	TK1	-1.56			
Cyclin dependent kinase 4	U79269	CDK4		-1.71	-3.11	
Cyclin D3	NM_001760	CCND3			-1.98	
Calpactin 1	M81457	S100A10				-2.32
<b>Cell differentiation</b>						
Keratin 19	NM_002276	KRT19	-1.52			
Osteoprotegrin	U94332	OPG	-2.34			
Inhibitor of DNA binding 1	X77956	ID1				-2.25
<b>Cell signalling / receptor</b>						
Transcription factor AP2-gamma	NM_003222	TFAP2C	-1.98	-1.88		
<b>Chromosomal processing</b>						
Histone 4 member M consensus	NM_003495	H4FM		-1.87	-6.76	-2.49
Histone 3 member F consensus	NM_003533	H3FF				-1.86
<b>DNA repair / synthesis</b>						
Ribonucleotide-reductase M1	NM_001033	RRM1	-2.16			
Polysynthetase	J03473	ADPRT	-3.07		-2.40	
Thymidilate synthetase	NM_001071	TYMS			-4.11	-2.17
<b>Extracellular matrix / Cell structure</b>						
Collagen I-alpha 1	NM_000088	COL1A1	-1.89	-1.81		-1.70
Collagen III-alpha 1	NM_000090	COL3A1	-3.56	-3.71		
Collagen VI-alpha 2	NM_001849	COL6A2	-2.03	-2.35		
Tissue inhibitor of metalloproteinase 1	NM_003254	TIMP1	-0.13			
Matrix metalloproteinase 1	NM_002421	MMP1	-1.51			
Cell division cycle 42	NM_001791	CDC42				-1.65
<b>Growth factor and cytokin</b>						
Vascular endothelial growth factor C	NM_005429	VEGFC	-1.88		-1.73	
Interleukin 1B	M15330	IL1B	-2.00			
Connective tissue growth factor	U14750	CTGF			-1.76	
Interleukin 8	NM_000584	IL8		-1.72		
<b>Intermediate / Protein metabolism</b>						
Ornithine decarboxylase	NM_002539	ODC			-2.04	
Creatin kinase brain	M16364	CKB			-1.79	
Nuclear RNA helicase	Z37166	BAT1	-1.64		-2.42	
Eukariotic translation initiation factor 4A	NM_001416	EIF-4A			-1.81	
Translation initiation factor 3 subunit 6	NM_001568	INT6				-3.26
<b>Oncogenesis/Tumor suppressor</b>						
c-raf1	X03484	RAF1	-1.59			
Ewing sarcoma breakpoint region 1	NM_005243	EWSR1			-2.44	-1.88
TGFβ RcII	D50683	TGFBR2	-1.94			
<b>Proteasome</b>						
Proteasome 26S	AB003102	PSMD11	-1.54			
Proteasome subunit	NM_002788	PSMA3				-2.87
<b>Stress response</b>						
Superoxide dismutase	NM_000636	SOD2	-5.10	-1.77		
Methionin sulfoxide reductase A	AF183420	MSRA	-2.08			
Heme oxygenase	NM_002133	HMOX			-1.82	
Apolipoprotein J	J02908	APOJ		-4.34		
Heat shock 90kD protein1 alpha	X15183	HSP90-α			-2.14	
Metallothionein 2A	V00594	MT2A				-2.16

3 Table 2 (A) and (B): List of genes with increased (A) and decreased (B) transcript level in FF95  
4 human skin fibroblasts after PUVA treatment (Column 1 = gene names grouped by function,  
5 column 2 = GenBank accession number). Total RNA was collected at 24 h, 72 h, 1 week and 4  
6 weeks after PUVA treatment. Empty boxes = level unchanged.

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8

1 Table 3 (A) Genes overexpressed (control=1.00)

Name	Genbank	Genes	24 h	72 h	1 week	4 weeks
<b>Apoptosis / Inflammation</b>						
Bcl2 associated X protein	NM_004324	BAX	1.65	1.53		
BCLX	NM_001191	BCLX	2.24			
<b>Cell cycle</b>						
Cyclin G	U53328	CCNG	2.02	2.17		2.07
Cyclin dependent kinase inhibitor 2A	L27211	p16				2.71
Cyclin dependent kinase inhibitor 1A	U03106	p21	3.36	3.57	2.60	3.30
Cyclin D1	NM_053056	CCND1		2.77		
Proliferating cell nuclear antigen	NM_002592	PCNA	3.63	4.36		
Cyclin D3	NM_001760	CCND3	1.63			
<b>Cell differentiation</b>						
Keratin 19	NM_002276	KRT19		1.81		
Sorcin	NM_003130	SRI	1.79			
<b>Cell signaling receptor</b>						
Urokinase receptor	NM_002659	PLAUR	1.63			
<b>Chromosomal processing</b>						
Histone 2b member B/S consensus	NM_080593	H2B/S	2.00	4.22		2.37
<b>Circulation</b>						
Plasminogen activator tissue	NM_000930	TPA	1.72	2.15		
<b>DNA repair / synthesis</b>						
Thymidylate synthetase	NM_001071	TYMS	1.93			
<b>Extracellular matrix / Cell structure</b>						
Matrix metalloproteinase 1	NM_002421	MMP1	5.03	3.96		
Matrix metalloproteinase 14	NM_004995	MMP14	1.89	2.25	1.72	
Cell division cycle 42	NM_001791	CDC42	1.75	2.13		
<b>Growth factor and cytokin</b>						
TGF $\beta$ 1	NM_000660	TGFB1	1.55			
<b>Intermediate / Protein metabolism</b>						
Electron transfer flavoprotein beta	NM_001985	ETFB				1.76
Pyruvate kinase muscle	M26252	PKM2		1.70		
Ornithin decarboxylase 1	NM_002539	ODC	2.43	1.83		
$\beta$ 1 galactosidase	M34423	GLB1	1.54			
Translation initiation factor 3 subunit 6	NM_001568	INT6	1.99	1.77		
60S-ribosomal protein L3	NM_000967	RPL3		1.58		
Eukaryotic translation initiation factor 4A	NM_001416	EIF-4A	1.86	1.61		
Nuclear RNA helicase	Z37166	BAT1	1.61	1.74		
Eukaryotic translation elongation factor A1	AY043301	EF1A		3.03		
Calnexin	NM_001746	CANX	1.41	1.68		
Cathepsin H	NM_004390	CTSH	1.61			
<b>Oncogenesis</b>						
SHC transforming protein 1	U73377	SHC	1.67	1.71		1.68
R-ras	NM_006270	RRAS				2.12
Calgizzarin	D38583	S100A11		1.75		
Ewing sarcoma breakpoint region 1	NM_005243	EWSR1	1.64			
<b>Proteasome</b>						
Proteasome (prosome, macropain) 36S subunit	NM_002807	PSMD1		2.76		
Proteasome (prosome, macropain) subunit 2	NM_002787	PSMA2		2.15		
Proteasome (prosome, macropain) subunit 3	NM_002788	PSMA3	2.01	1.99		
Proteasome 26S	AB003102	PSMD11	1.84			
<b>Stress response</b>						
Heme oxygenase	NM_002133	HMOX	3.82	1.56		
Anti oxidant protein 2	NM_004905	AOP2		1.59		
Metallothionein 2A	V00594	MT2A		2.00		
Heat shock 90kD protein1 alpha	X15183	HSP90- $\alpha$	1.77	3.33		
Heat shock 70kD protein1	AB023420	HSP70	1.83			
Heat shock 40kD protein1	D49547	HSP40	1.54			
Annexin 1	NM_000700	ANX1	1.89	1.62		
Glutathione S transferase theta 1	NM_000853	GSTT1				1.99
Glutathione peroxidase	M21304	GPX		1.96	2.31	2.78
Superoxide dismutase	NM_000636	SOD2		3.64	3.83	6.17
DNA damage inducible transcript 3	S40706	GADD153				2.27

2

## 1 (B) Genes down-regulated (control=1.00)

Name	Genbank	Genes	24 h	72 h	1 week	4 weeks
<b>Cell signaling/receptor</b>						
Interleukin1 receptor-alpha	U32324	IL11RA		-1.47		
<b>Chromosomal processing</b>						
Histone 4 member M consensus	NM_003495	H4FM	-2.12			
<b>Cell adhesion / Circulation</b>						
Plasminogen activator inhibitor type 1	M14083	PAI1		-1.47		-3.42
<b>Extracellular matrix / Cell structure</b>						
Matrix metalloproteinase 1	NM_002421	MMP1				-6.29
Collagen I alpha 1	NM_000088	COL1A1		-2.23	-2.22	
Collagen III alpha 1	NM_000090	COL3A1		-2.31		
Osteonectin	NM_003118	Oste		-1.59	-2.10	
Fibronectin	X02761	FN1		-1.73		
Transgelin	M95787	SM22		-2.04	-1.89	
<b>Growth factor and cytokin</b>						
Insulin growth factor binding protein 3	X64875	IGFBP3	-1.83	-2.16	-2.78	-2.27
Insulin growth factor binding protein 5	M65062	IGFBP5		-1.22		
Connective tissue growth factor	U14750	CTGF		-2.26	-2.50	
<b>Stress response</b>						
Metallothionein 2A	V00594	MT2A				-4.51
Heat shock 27kD protein 1	AB020027	HSP27		-1.60		

- 2 Table 3 (A) and (B): List of genes with increased (A) and decreased (B) transcript level in  
3 AG04431 human skin fibroblasts after PUVA treatment (Column 1 = gene names grouped by  
4 function, column 2 = GenBank accession number). Total RNA was collected at 24 h, 72 h, 1  
5 week and 4 weeks after PUVA treatment. Empty boxes = level unchanged.

1 Table 4 (A) Genes overexpressed (control=1.00)

Name	Genbank	Genes	24 h	72 h	1 week	4 weeks		
			<b>9. F</b>	<b>10. F</b>	<b>11. F</b>	<b>12. F</b>		
			<b>F</b>	<b>F</b>	<b>F</b>	<b>F</b>		
			<b>9</b>	<b>9</b>	<b>9</b>	<b>9</b>		
			<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>		
			AG04431	AG04431	AG04431	AG04431		
<b>Apoptosis / Inflammation</b>								
BCLX	NM_001191	BCLX	2.24	<b>2.14</b>	<b>2.40</b>	<b>1.97</b>	<b>2.42</b>	
<b>Cell cycle</b>								
Cyclin G	U53328	CCNG	2.02	2.17		<b>2.00</b>	2.07	
Cyclin D1	NM_053056	CCND1		2.77	<b>2.06</b>		<b>2.73</b>	
Cyclin dependent kinase inhibitor 1A	U03106	p21	3.36	<b>9.77</b>	3.57	<b>4.26</b>	<b>2.78</b>	
Proliferating cell nuclear antigen	NM_002592	PCNA	3.63	<b>1.84</b>	4.36	<b>2.15</b>		
<b>Cell differentiation</b>								
Keratin 19	NM_002276	KRT19		1.81	<b>2.32</b>			
<b>Cell signaling receptor</b>								
Urokinase receptor	NM_002659	PLAUR	1.63		<b>1.71</b>			
<b>Chromosomal processing</b>								
Histone2b member B/S consensus	NM_080593	H2B/S	2.00	<b>1.60</b>	4.22		<b>2.37</b>	<b>4.43</b>
<b>Extracellular matrix</b>								
Matrix metalloproteinase 14	NM_004995	MMP14	1.89	2.25	1.72		<b>2.97</b>	
<b>Growth factor and cytokin</b>								
TGF B1	NM_000660	TGFB1	1.55	<b>2.02</b>				
<b>Intermediate / Protein metabolism</b>								
Ornithin decarboxylase 1	NM_002539	ODC	2.43	1.83	<b>3.21</b>			
B1 galactosidase	M34423	GLB1	1.54			<b>1.79</b>	<b>2.05</b>	
Cathepsin H	NM_004390	CTSH	1.61				<b>5.92</b>	
<b>Oncogenesis</b>								
SHC transforming protein 1	U73377	SHC	1.67	1.71		<b>1.98</b>	1.68	
R-ras	NM_006270	RRAS				<b>2.52</b>	2.12	
<b>Stress response</b>								
Heme oxygenase	NM_002133	HMOX	3.82	<b>2.88</b>	1.56	<b>2.60</b>	<b>2.13</b>	
Anti oxidant protein 2	NM_004905	AOP2			1.59	<b>1.59</b>		
Heat shock 90kD protein1 alpha	X15183	HSP90- $\alpha$	1.77		3.33	<b>2.07</b>		
DNA damage inducible transcript 3	S40706	GADD153		<b>4.32</b>		<b>2.35</b>		
Glutathione S-transferase theta 1	NM_000853	GSTT1				<b>1.57</b>	1.99	
Glutathione peroxidase	M21304	GPX	<b>2.33</b>	-1.96	<b>1.80</b>	2.31	1.75	
Superoxide dismutase	NM_000636	SOD2		3.64	<b>1.80</b>	3.83	<b>17.74</b>	6.17
Annexin 1	NM_000700	ANX1	1.89	1.62	<b>2.69</b>			

2

3 Table 4 (B) Genes down-regulated (control=1.00)

Name	Genbank	Genes	24 h	72 h	1 week	4 weeks	
				<b>13. F</b>	<b>14. F</b>		
				<b>F</b>	<b>F</b>		
				<b>9</b>	<b>9</b>		
				<b>5</b>	<b>5</b>		
			AG04431	AG04431	AG04431	FF95	
<b>Chromosomal processing</b>							
Histone 4 member M consensus	NM_003495	H4FM	<b>-2.12</b>	-1.87	-6.76	-2.49	
<b>Extracellular matrix</b>							
Collagen I-alpha 1	NM_000088	COL1A1	-1.89	-1.81	<b>-2.23</b>	<b>-2.22</b>	-1.70
Collagen III-alpha 1	NM_000090	COL3A1	-3.56	-3.71	<b>-2.31</b>		
Matrix metalloproteinase 1	NM_002421	MMP1	-1.51				<b>-6.29</b>
<b>Growth factor and cytokin</b>							
Connective tissue growth factor	U14750	CTGF			<b>-2.26</b>	-1.76	<b>-2.50</b>
<b>Stress response</b>							
Metallothionein 2A	V00594	MT2A				-2.16	<b>-4.51</b>

4

5 Table 4 (A) and (B): List of genes with increased (A) and decreased (B) transcript level in both  
6 strains of fibroblasts (AG04431-FF95) (Column 1 = gene names grouped by function, column 2  
7 = GenBank accession number). Total RNA collected 24 h, 72 h, 1 week and 4 weeks after PUVA  
8 treatment. Empty boxes = level unchanged.

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<b>p21<sup>WAF-1</sup></b>	<b>FF95</b>		<b>AG04431</b>	
	Real Time RT-PCR	cDNA Array	Real Time RT-PCR	cDNA Array
24 hours	32.2	9.8	6.5	3.4
72 hours	13.7	4.3	18.8	3.6
1 week	10.2	5.0	6.0	2.6
4 weeks	8.3	2.8	3.5	3.3

2

2.5. <b>HMOX</b>	<b>FF95</b>		<b>AG04431</b>	
	Real Time RT-PCR	cDNA Array	Real Time RT-PCR	cDNA Array
24 hours	4.9	2.9	2.1	3.8
72 hours	2.4	2.6	1.9	1.6
1 week	-2.0	-1.8 (N.S.)	-1.2 (N.S.)	1.3 (N.S.)
4 weeks	2.5	2.1	1.1 (N.S.)	-1.1 (N.S.)

3

<b>CTGF</b>	<b>FF95</b>		<b>AG04431</b>	
	Real Time RT-PCR	cDNA Array	Real Time RT-PCR	cDNA Array
24 hours	1.1 (N.S.)	-1.3 (N.S.)	-2.9	-1.5 (N.S.)
72 hours	1.4	1.8 (N.S.)	-1.3	-2.2
1 week	-1.4	-1.7	-3.3	-2.5
4 weeks	1.2 (N.S.)	4.4 (N.S.)	1.2 (N.S.)	-1.8 (N.S.)

4

<b>MT2A</b>	<b>FF95</b>		<b>AG04431</b>	
	Real Time RT-PCR	cDNA Array	Real Time RT-PCR	cDNA Array
24 hours	-4.1	-1.2 (N.S.)	-1.3	-1.8 (N.S.)
72 hours	-1.5	-1.5 (N.S.)	5.7	2.0
1 week	-3.4	-1.5 (N.S.)	-1.8	-1.4 (N.S.)
4 weeks	-4.4	-2.2	-1.5	-4.5

5

<b>SOD2</b>	<b>FF95</b>		<b>AG04431</b>	
	Real Time RT-PCR	cDNA Array	Real Time RT-PCR	cDNA Array
24 hours	-8.2	-4.8 (N.S.)	-2.0	-1.1 (N.S.)
72 hours	-2.0	-1.8 (N.S.)	3.5	3.5
1 week	23.4	17.7	4.1	3.8
4 weeks	3.3	2.0	7.5	6.2

6

7 Table 5 : Comparison between the data obtained with real-time RT-PCR and the DualChip  
8 human aging for FF95 fibroblasts and AG04431 fibroblasts for 5 genes at 24 h, 72 h, 1 week and  
9 4 weeks after PUVA treatment (N.S.: non significant), cut off : 1,3.

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