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Ageing effects on the expression of cell defence genes after UVA irradiation in human male cutaneous fibroblasts using cDNA arrays

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Abstract

Ageing is a multifactorial process in which reactive oxygen species (ROS) are thought to be implicated. ROS cause oxidative alterations on cell constituents, and damage accumulation can lead to mutations in DNA. Modulation of gene expression during ageing is now quite documented but results are often controversial and/or incomplete. As ultraviolet A is one of the exogenous factors involved in skin ageing, by the production of ROS, we further document the modifications in gene expression during ageing process and response to an oxidative stress. For this purpose, we used a cDNA macroarray containing 82 genes related to cell defence, essentially represented by antioxidant and DNA repair proteins. Ageing-associated gene expression was assessed in normal skin human fibroblasts from three age groups: children ($n = 4$), adults ($n = 4$) and olders ($n = 3$), at the basal state and after a 5 J/cm^2 UVA irradiation. Analysis revealed that 22 genes were never detected, whereas certain were always expressed such as those related to antioxidant defence, extracellular matrix (ECM) regulator and XPC. Transcripts related to ECM, MMP1 and MMP3 were increased with age and after UVA irradiation, independently of age. It appeared that transcripts involved in the redox status control (TXN and APEX) decreased as a function of age, at the basal state and after irradiation, respectively. Most of transcripts involved in DNA repair were not detected but repression of POLD1 in the adult group and induction of XRCC5 and LIG4 were observed after UVA irradiation, as a function of age. In the basal state, the transcript of GAS1, regulator of cell cycle arrest in G1 phase was found to be decreased with age. HMOX1 increased after UVA irradiation. In conclusion, the decrease in expression of some antioxidant system, cell cycle control gene and extracellular matrix enzymes, particularly after UV exposure can explain the occurrence of photoaging.

Keywords: Ageing; UVA; cDNA array; Male skin fibroblasts; DNA repair; Redox and antioxidant status

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1. Introduction

Ageing is a multifactorial complex process but there are great evidences that accumulation of oxidative damage to DNA and alterations in gene regulation are two essential features of this phenomenon.

There is strong evidence that photoaging is established as an abnormal consequence of chronic exposure of human skin to solar irradiation. Ultraviolet A (UVA, 400–315 nm) particularly contributes to ageing at the skin level [1] by penetrating until dermis [2] and interacting with chromophores to produce reactive oxygen species (ROS). ROS cause molecular damage to proteins [3,4], lipids [5] and nucleic acids [6].

Fortunately, numerous defence systems protect cellular macromolecules against oxidation. Among them, antioxidant molecules such as glutathione or vitamins scavenge free radicals. Antioxidant enzymes such as superoxide dismutase (SOD) which reacts with superoxide anion ($O_2^{\bullet -}$) and catalase which reacts with H_2O_2 are very important for the detoxification of cells from ROS. DNA repair systems and particularly base excision repair (BER) take charge of oxidized bases, abasic sites and single strand breaks generated by oxidative processes. However, cell defences against oxidative stress are also suspected to decrease with age mainly through changes in gene expression in response to oxidative stress [7]. Response to UVA irradiation is complex and involves the participation of different classes of genes.

The expression levels of genes can be quantified using a cDNA microarray containing probes related to genes of interest. This method is powerful and very sensitive [8,9]. Recently, microarrays have been largely used in studies of ageing. Most of them focused on ageing in the skeletal muscle [10–13] and in brain [14–16]. Few data concerned skin ageing and fibroblasts except the one published by Shelton et al. [17] who evaluated gene expression in dermal fibroblasts submitted to replicative senescence. Results are often discordant. Nevertheless, during ageing the common features are that expression of genes related to cell defences and inflammation increased, whereas expression of genes related to energetic metabolism decreased. Studies of gene expression must help to better understand the function of genes, and to determine the network of genes involved in such a process.

In this study, we explored the response to oxidative stress with ageing using the transcriptional profile of genes in primary skin fibroblasts at the basal state and after application of the stress. As UVA is an important exogenous factor involved in cutaneous ageing, we chose it as the oxidative stress and we selected skin fibroblasts from male donors of different ages, as cellular model. We constructed a cDNA microarray, containing 82 probes including gene families involved in cell defence to oxidative stress and DNA repair. We analysed gene expression levels via expression of their transcripts.

2. Materials and methods

2.1. Cell culture and UVA irradiation

Primary human dermal fibroblasts were prepared from excess tissues obtained from eleven non-exposed normal Caucasian skin as described [18]. All the donors were male. Three classes of age were determined: four children aged 2–5 years (mean age 3.25 years), four adults aged 24–35 years (mean age 28 years) and three olders aged 74–82 years (mean age 78 years).

Cells were cultured in RPMI 1640 without L-glutamine (Invitrogen Life Technology, Cergy Pontoise, France), enriched with a mix of L-glutamine 200 mM, penicillin (10,000 U/ml), streptomycin (10 mg/ml), kanamycin (50 mg/ml) (all from Sigma Cell Culture, Courtaboeuf, France), amphotericine B (0.5 mg/ml) (Fungizone® 50 mg, Bristol Myers Squibb, Puteaux, France) and with 15% fetal serum bovine (FBS E.U approved origin, Gibco BRL, Life Technology, Grand Island, NY, USA) in humidified incubators at 37 °C enriched in 5% CO_2. Fibroblasts were passaged each week (ratio 1:2).

10^6 cells semi-confluent cells were subcultured in 75 cm^2 flask and were exposed to 5 J/cm^2 UVA irradiation, delivered from Tecimex Apparatus (Dixwell, St Symphorien d'Ozon, France) with λ_{max} at 372 nm and the relative spectral distribution of the lamp has been described [19]. Cells were harvested 6 hours after UVA irradiation, centrifuged (300*g*, 3 min, room temperature) and rinsed two times with PBS (Dulbecco's phosphate buffered saline, Sigma, Courtaboeuf, France). Then, pellets were rapidly frozen in liquid nitrogen and placed at −80 °C until total RNA extraction was performed.

2.2. Choice of genes

Genes are represented by 82 cDNA clones corresponding to the 3′ end of the mRNA and were obtained through the Image Consortium at the National Center for Biotechnology Information (NCBI). Clones were almost all selected from libraries constructed by B Soares and had to fulfill certain criteria such as homogenous insert size (between 500 and 1200 bases pairs (bp)) and absence of detectable repeated sequences (Table 1).

Families chosen were antioxidant enzymes ($n = 21$), DNA repair ($n = 32$), tumor suppressive genes or associated family ($n = 9$), protooncogene family or associated ($n = 7$), extracellular matrix related genes ($n = 5$), inflammatory or immune response family ($n = 3$) and house keeping genes ($n = 5$). Total number of ESTs was $n = 82$. The names, accession number of the mRNA and of the clone, the UniGene number, the description and the gene function are indicated in Table 2.

We included positive and negative controls as described: negative control were 3 poly(A) sequences 50

Table 1
Websites used for screening databases (with a given genomic segment) and for assessing sequences information generated

Name	Web site
UniGene	http://www.ncbi.nlm.nih.gov/
Image Consortium	http://bbrp.llnl.gov/bbrp/image/image.html
Genomic Data Bank	http://gdbwww.gdb.org
Blast	http://www.ncbi.nlm.nih.gov/BLAST/

pb, 60 pb and 90 pb long and the empty cloning vector pT7T3 D-Pac. Positive control was a plant gene (GCO3 gene of *Arabidopsis thaliana*) used to monitor reverse-transcription efficiency, hybridization quality and gene position on the array.

2.3. Bacteria culture

Bacteria were cultured in 5 ml Luria Bertani (LB) medium 1× (from LB 10×: 10% bactopeptone, 5% Yeast Extract, 10% NaCl, all from Sigma) complemented with Ampicillin (50 μg/ml), 37 °C, 200 rpm, in a shaking incubator during 12 h. The bacterial suspension was centrifuged 10 min at 4 °C, 4000*g* and the pellet was resuspended in 10 ml sterile water to lyse bacteria and release the plasmid clones. 10 μl of this suspension were used for EST amplification.

2.4. EST amplification: PCR

The cDNA clones were amplified in a reaction mixture (all from Eurobio, Les Ulis, France) composed of 1× PCR buffer, 1.5 mM $MgCl_2$, 0.5 μM LBP1S forward primer (5′GTGGAATTGTGAGCGGATAACA3′), 0.5 μM LBP1AS reverse primer (5′GCAAGGCGATTAAGTTGGG3′), both primers from Genome Express, Meylan, France), 1.25 mM dNTPs mixture and 5 U Taq in MilliQ water. Reactions were carried out in a thermocycler (Crocodile, Appligene, France) using the following cycling program: 94 °C-6 min, (94 °C-30 s, 48 °C-1 min, 72 °C-1 min) for 40 cycles, and 72 °C-10 min.

The identity of each clone was checked by the size of PCR product, by migration on 1.5% agarose gel (60 min, 100 mA) with molecular weight markers (XIV, Gibco BRL, Invitrogen Life Technologies, Cergy-Pontoise, France).

Finally, PCR products were purified (Microcon YM-50, Millipore, Bedford, MA, USA), quantified (Low DNA Mass Ladder, Invitrogen Life Technologies, Cergy-Pontoise, France), after migration on 1% agarose gel, by the software Quantity-One (BioRad) and adjusted to a concentration of 200 ng/μl with MilliQ water.

2.5. Nylon microarray preparation

Microarray were prepared by spotting the PCR products (200 ng/μl) onto nylon N+ membranes (Hybond + Amersham Biosciences, Orsay, France) using the solid-pin micro spotting arrayer GMS 417 (MWG Biotech, Courtaboeuf, France). Each spot was distant by 500 μm and the volume of PCR product per spot was 400 pl (4 hits per spot, 1 hit = 100 pl). After spotting, nylon membranes were first denatured on a Schleicher & Schuell GB 003 paper (VWR Merck Eurolab, Strasbourg-Cronenbourg, France) saturated with 1.5 M NaCl, 0.5 M NaOH and thereafter neutralized with 1.5 M NaCl, 0.5 M Tris–HCl, 1 mM EDTA. Finally, a UV treatment crosslinked the DNA to the membranes (UVC, 0.250 J, UVItec, VWR, Strasbourg-Cronenbourg, France).

2.6. Total RNA extraction

Total RNAs were extracted from fibroblast pellets (about 1.10^6 cells), with RNeasy® Mini kit (Qiagen, Saint Louis, USA) according to manufacturer recommendations. Briefly, cells were lysed and nucleic acids were isolated by the selective binding properties of a silica-gel based membrane. Total RNA was eluted with 50 μl of RNase free water. RNA quality and yield were evaluated after agarose gel electrophoresis under standard conditions and spectrophotometer measurement.

2.7. cDNA synthesis and radioactive labeling

Labeled cDNAs were prepared from total RNA by reverse transcription with incorporation of [α^{33}P]dCTP and oligo dT_{25} as a primer (Invitrogen Life Technologies, Cergy Pontoise, France).

Total RNA (5 μg per reaction) was treated by DNAse according to the manufacturer recommendations (DNase I Amplification Grade Invitrogen Life Technologies, Cergy-Pontoise, France).

Annealing of mRNA and 0.25 ng internal control RNA CGO3, from *Arabidopsis thaliana* (given by INSERM 318, La Tronche) was performed with 8 μg of dT_{25} (Genome Express, Meylan, France) during 5 min at 70 °C and progressively cooled to 42 °C.

The reverse transcription was carried out in a reaction mixture containing 40 U RNasin (RNasin® Ribonuclease Inhibitor, Promega, Madison, USA), 30 μCi [α^{33}P]dCTP (Amersham Biosciences, Orsay, France) with 0.4 mM unlabeled dATP, dTTP and dGTP, 2.4 μM dCTP (all from PCR Grade, Roche, Meylan), 0.1 M DTT (Invitrogen Life Technologies, Cergy Pontoise, France), 1.6× reverse transcription buffer (5× Invitrogen Life Technologies, Cergy Pontoise, France) and 200 U SuperScript™ II RNase H^- (Life Technologies, Cergy Pontoise, France), during 2 h at 42 °C.

The unincorporated nucleotides were removed using an exclusion column (YM-50, Millipore, Bedford, MA, USA) and the heteroduplexes cDNA-RNAm were resuspended in 100 μl sterile water. Reverse transcrip-

Table 2
Alphabetical list of genes per family: antioxidant family ($n = 21$), DNA repair family ($n = 32$), tumor suppressive or associated family ($n = 7$), caspase family ($n = 2$), extracellular matrix regulator family ($n = 5$), inflammatory or immune response family ($n = 3$), protooncogene family or associated and ageing related family ($n = 7$) and house keeping genes ($n = 5$)

Gene	Accession Number RNAm	Accession Number	Clone ID	Description	Function
AOE372/PRDX4	NM_006406	AI911758	2329821	Thioredoxin peroxidase	Antioxidant enzyme involved in redox regulation of the cell
CAT	NM_001752	AI076597	1676912	Catalase	Scavenger of hydrogen peroxyde
FBP1	NM_000507	AI6774572	2314081	Fructose-16-biphosphatase 1	Gluconeogenesis regulatory enzyme catalyzes the hydrolysis of fructose 1,6 BP to F6P + Pi
GLCLC	NM_001498	AI760976	2398721	Glutamate-cysteine ligase (gamma-glutamylcysteine synthetase), catalytic (72.8kD)	Involved in glutathione synthesis
GLCLR	NM_002061	AA931744	1572218	Glutamate-cysteine ligase (gamma-glutamylcysteine synthetase), regulatory (30.8kD)	Involved in glutathione synthesis
GPX1	NM_00581	H98823/N23548	261716	Glutathione peroxidase 1	Detoxification of hydrogen peroxyde
GPX1	NM_00581	AI218741	1565472	Glutathione peroxidase 1	Detoxification of hydrogen peroxyde
GSR	NM_000637	AA574223	1056796	Glutathion reductase	Reduction of glutathione
GSS	NM_000178	AI800712	2063720	Glutathione synthetase	Involved in glutathione synthesis
GSTA4	NM_001512	AI125246	1736518	Glutathione S-transferase A4	Cellular defence against toxic carcinogenesis and pharmacatolly active Electrophilic compounds
GSTM5	NM_000851	AI239656	1846389	GlutathioneS-transferase M5	Cellular defence against toxic carcinogenesis and pharmacatolly active Electrophilic compounds
HMOX1	NM_002133	AW001610	2521989	Heme oxygenase 1	Involved in heme catabolism. Cleaves heme to form biliverdin. Inductible
HMOX2	NM_002134	AI889030	2428794	Heme oxygenase 2	Constitutive heme oxygenase
MPO	NM_000250	AI056254	1673143	Myeloperoxydase	Heme protein synthesis during myeloid differenciation. Involved in the Microbicidal activity of neutrophyles
SEPW1	NM_003009	AW085571	25553615	Selenoprotein W	Involved in oxidation/reduction reactions
SOD1	NM_000454	AI 040245	1663768	ZnCuSOD	Destroys radicals. Cytoplasmic
SOD2	NM_000636	AA011435	429667	MnSOD mitochondrial	Destroys radicals. Mitochondrial sub cellular location
SOD3	NM_003102	AI015650	1635541	ZnCuSOD extracellular	Destroys radicals. Extracellular location
TXN	NM_003329	W95350/W95392	358157	Thioredoxin	Redox control and defence against oxidative stress
TXNL	NM_004786	A885166	2432194	Thioredoxin-like	Acts as thioredoxin

TXNRD1	NM_00330	R93223	196602	Thioredoxin reductase	Including thioredoxin and NADPH. Catalyses the transfer of electrons from NADPH to TXN, acts as a reductant of disulfide-containing proteins.
ADPRTPARP1	NM_ 001618	AA421106/AA397988	729238	Poly ADP-ribose polymerase transferase	Involved in the BER pathway following DNA damage. Important step in a detection signaling pathway
APC	NM_000038	AW000963	2495009	Adenomatous polyposis coli protein	Control progression through mitosis and the G1 phase of the cell cycle
APEX	NM_001641	AA877644	1160654	AP endonuclease	Repair oxidative DNA damage. May have a role in protection against cell lethality and suppression of mutations
ATR FRP1	NM_001184	AI127664	1710334	Ataxia telangiectasia and RAD3 related Frap related	Related to ATM, a protein kinase encoded by the gene mutated in ataxia telangiectasia
BRCA1	NM_007297	AI217721	1844798	Breast Cancer 1 early onset	Tumor suppressor in human breast cancer. Mutations to BRCA1 are predicted to be responsible for some breast and ovarian cancer
CKN1/CSA 1	NM_000082	AI078139	1676809	Cockayne syndrome 1	Involved in transcription. Defects in CKN1 are the cause of Cockaine's syndrome type A
ERCC1	NM_001983	AI143627	1705819	Excision repair cross-complementing rodent repair deficiency, complementation group 1	DNA repair endonuclease responsible for the 5′ prime incision during DNA repair
ERCC2/XPD	NM_000400	AI918117	2166805	Homo sapiens excision repair cross-complementing rodent repair deficiency, complementation group 2 (xeroderma pigmentosum D)	Corrects defective DNA strands. Break repair and sister Chromatid exchange following treatment with ionizing radiation and alkylating agents.
ERCC3/XPB	NM_000122	AI806901	2357699	Excision repair cross-complementing rodent repair deficiency, complementation group 3 (xeroderma pigmentosum group B complementing)	ATP-dependent 3′-5′ DNA helicase involved in NER of DNA
ERCC4/XPF	NM_005236	AA770518	1321363	Homo sapiens excision repair cross-complementing rodent repair deficiency, complementation group 4	DNA repair endonuclease responsible for the 5′ prime inciseduring DNA repair. Involves in homologous recombination

(*continued on next page*)

Table 2 (*continued*)

Gene	Accession Number RNAm	Accession Number	Clone ID	Description	Function
ERCC5/XPG	NM_000123	AI417946	2112719	Excision repair cross-complementing rodent repair deficiency, complementation group 5 (xeroderma pigmentosum, complementation group G (Cockayne syndrome)	DNA repair endonuclease responsible for the 3′ incision in NER
GTF2H1/p62	NM_005316	AI636101	2296094	General transcr. factor IIH pept 1(62 kD)	Component of the core-TFIIH, involved in NER
GTF2H4/p52	NM_001517	AI332776	1931028	General transcr.factor IIH pept 4(52 kD)	
Ku80/XRCC5	NM_021141	W52910/AA037353	321207	X-ray Repair Cross-complementing defectice repair in Chinese hamster cells 5	Works in the 3′-5′ direction
LIG1	NM_000234	AW024287	2512719	DNA ligase 1	It seals during DNA replication, recombinaison and repair
LIG4	NM_002312	R54358	39274	LIG4 DNA ligase IV	Essential for V(D)J recombinaison and DSB, repair through NHEJ
MPG glycosidase	NM_002434	AI209171	1760130	DNA-3-methyladenine glycosidase	Hydrolysis of the deoxyribose N-glycosidic bond to excise 3-methyl adenine and 7 methylguanine from the damaged DNA polymers formed by alkaline lesions
MSH2/COCA2/FCC2	NM_00025	AI948713	2472255	DNA mismatch repair protein	Involved in DNA mismatch repair
MutYH glycosylase	NM_0122222	AW005872	2566061	MutYH glycosylase	Adenine-DNA glycosylase
OGG1 DNA-glycosylase	NM_002542 (variant 1a)	AA689454	1183918	8-Oxoguanine DNA-glycosylase	DNA repair enzyme that incises DNA at the 8-oxoG residues. Excises 8-oxodGuo and Fapy
POLD1	NM_002691	AI934491	2465996	DNA polymerase delta catalytic subunit 1	2 enzymatic activities: DNA synthesis and exonucleolytic activity that degrades single strand DNA in the 3′-5′ direction
POLD2	NM_ 006230	AI628642	2285116	DNA polymerase delta subunit 2	2 enzymatic activities: DNA synthesis and exonucleolytic activity that degrades single strand DNA in the 3′-5′ direction
RAD23B HHR23B/p58	NM_002874	AA628957	1033020	Complementing protein for XPC	Involved in DNA excision repair. May play a part in DNA damage recognition and/or altering chromatin structure to allow access by damage-processing enzymes

RAD51 RAD51ARECA	NM_002875	AA568782	1059434	Radiation sensitive abnormal 51	Reduced double-strand breaks-induced homologous recombinaison in mammalian cells
RAD52	NM_002879	AI056288	1674200	Radiation sensitive abnormal 52	DNA double-strand breaks repair and homologous recombinaison
RPA2 32 KD	NM_002946	AI870836	2430890	Replication protein 32 KD	Participates in a very early step in initiation. Single strand binding protein
RPA3 replication protein 14 KD	NM_002947	AI688239	2326236	Replication protein 14 KD	Heterodimer consisting of three subunits involved in DNA damage repair
TDG g/t	NM_003211	AI276095	1878108	Thymine-DNA glycosylase	Hydrolytic deamination of the 5′-methylcytosine to thymine leads to the formation of G/T mismatches. Corrects G/T mispairs to G/C pairs. It can remove thymine also from C/T and T/T mispairs in the order G/T>>C/T>T/T
UNG	NM_003362	AA707482	1291981	Uracil-DNA glycosylase	Excises uracil residues from the DNA which ca arise as a result of misincorporation of dUMP residues by DNA polymerase or due to deamination of cytosine
XPA	NM_000380	AI184821	1731890	Xeroderma pigmentosum complementation group A	Involved in DNA excision repair in initialising repair by binding to damaged sites with various affinities depending on the photoproduct and the transcriptional state of the region
XPC	NM_004628	W93782/W93783	357309	Xeroderma pigmentosum complementation group C	Initiator of global genomic NER
XRCC1	NM_006297	AW009176	2504069	X-ray Repair Cross-complementing defective repair in Chinese hamster cells 1	Thought to function as a scaffolding protein in both BER single strand break repair
DDIT3 GADD153	NM_004083	AW027012	2512884	DNA damage inducible transcript 3	Belongs to a subgroup of genes which are not rapidly induced by DNA damaging agents but are coordinately induced in growth-arrested cells
GADD45A	NM_001924	W93387/W94971	415112	Growth arrest-DNA damage inducible gamma	Growth arrest and DNA damage-inducible genes. Induced by p53 dependent or independent pathway.

(*continued on next page*)

Table 2 (*continued*)

Gene	Accession Number RNAm	Accession Number	Clone ID	Description	Function
GAS1	NM_002048	AI400635	2112151	Growth arrest specific 1	Specific growth arrest protein involved in growth suppression. Bock entry to S phase
GAS2	NM_005256	AI758363	2284211	Growth arrest specific 2	May play a role in apoptosis by acting as a cell death substrate for caspases. The cleaved form induces dramatic rearrangements of the actin cytoskeleton and potent changes in the shape of the affected cells.
MDM2	NM_002392	H13638	148052	Mouse Double Minute human homolog of p53 binding protein	It is a target gene of the transcription factor tumor protein p53. It is a nuclear phosphoprotein that binds and inhibits transactivation by tumor protein p53, as part of a autoregulatory negative feedback loop.
TP53	NM_000546	AI660717	2346084	p53	Nuclear protein plays an essential role in the regulation of the cell cycle, specifically in the transition G0 to G1. It is found in very low levels in normal cells, but it is expressed in high amount in a variety of transformed cell lines. Mutants of p53 frequently occur in a number of different human cancers.
PCNA	NM_002592	AI186133	1740919	Proliferative cell nuclear antigen	Cofacteur of DNA polymerase delta. Involved in DNA replication and following DBNA damage, protein is ubquitinated
CASP1	NM_001223	AI695109	2344212	Caspase 1	Member of the cysteine-aspartic acid protease family. Sequential activation of caspases plays a central role in the execution phase of cell apoptosis
CASP3	NM_004346	AI378787	2069645	Caspase 3	Was shown to cleave and activate caspases 6,7 and 9 and itself Could be processed by caspases 8, 9 and 10
ICAM-1	NM_000201	AI656039	2243477	InterCellular Adhesion Molecule 1 (CD54)	Typically expressed on endothelial cells and cells of the immune system. Binds integrins of type CD11a, CD18 or CD11B/CD18

MMP1	NM_002421	AI038497	1658571	Interstitial collagenase	Breakdown the interstitial collagens, types I, II, and III
MMP2	NM_004530	AI160943	1704954	72kD type IV collagenase gelatinase	Degrades type IV collagen, the major component of basement state
MMP3	NM_002422	AI281285	1873226	Stromelysin 1	Degrades fibronectin, laminin, collagens III, IV, IX and X. Thought to be involved in wound repair, progression of atherosclerosis and tumor initiation
MMP7	NM_002423	AI095584	1697164	Matrilysin	Degrades proteoglycans, fibronectin, elastin and casein. Wound Repair healing
GRO1	NM_001511	W46900	324437	Melanoma growth stimulating activity alpha	Neutrophil chemokines. Involved in inflammation resolution
IL15	NM_000585	AI379606	2068820	Interleukin 15	Cytokine that regulates T and natural killer cell activation and proliferation
MCP1	NM_002982	AA047236/AA04799	488534	Monocyte chemotactic protein SCYA2	Displays chemotactic activity for monocytes and basophyles. Implicated in the pathogenesis of disease characterized by Monocytes infiltration, like psoriasis.
CCNH/Cyclin CAK	NM_001239	AA902797	1519148		Component of TFIIH as well as RNA polymerase II protein complexes. Involved in cell cycle machinery
CROC4	NM_006365	AI6556811	2244561	c-fos transcription activator 4	Specific transcriptional activator of c-fos promotor
FOSB	NM_006732	AI217506	1845251	FBJ murine osteosarcoma viral oncogene homolog B	Gene encodes leucine zipper proteins that can dimerize with proteins of the JUN family, thereby forming the transcription factor complex AP-1. Implicated as regulators of cell proliferation, differentiation and transformation
UVARG	NM_003369	AI964046	2514529	UV radiation resistance associated gene	Gene involved in UV radiation resistance
SHC1 p66 SHCSHCA	NM_003029	AI185833	1740808	Src homology 2 domain containing	Involved in ageing process and signaling pathway. Induces elevation of extracellular oxidants, cytochrome c release and apoptosis

(*continued on next page*)

Table 2 (*continued*)

Gene	Accession Number RNAm	Accession Number	Clone ID	Description	Function
TEP1	NM_007110	AW025109	2525515	Telomerase associated protein	Component of the ribonucleoprotein complex responsible for telomerase activity which catalyses the addition of nem telomeres on the chromosome ends
TERT	NM_003219	AA748707	1270592	Telomerase reverse transcriptase	Maintains telomere ends by addition of the telomere repeat TTAGGG
ACTB	NM_001101	R97461/R97417	199520	beta-actin	Is one of the different actins isoforms which have been identified. Involved in cell motility, structure and integrity
GAPD	NM_002046	AA021601/AA021106	364038	Glyceraldehyde-3-phosphate dehydrogenase	Catalyzes an important energy yielding step in carbohydrate metabolism
HPRT	NM_000194	AA774427	1343887	Hypoxanthine-guanine phosphoribosyltransferase	Involved in the purine metabolism. Severe deficiency cause Lesh-Nyhan syndrome
RPL32	NM_000994	AI193187	1741568	Ribosomal protein 32	Protein belonging to ribosomes located in the cytoplasm
PLA2G5	NM_000929	AI051138	1669164	Phospholipase A2, group V	Ubiquitously expressed enzyme with key role in intracellular signaling, by releasing arachidonic acid from membranes phospholipids

Are also reported the names of the genes, the accession number of the mRNA, the accession number of the clone chosen, the UniGene number, the description and the gene function.

tion efficiency was checked by radioactive counting of 1 μl of purified product on Ready Cap™ (Beckman Coulter, Fullerton, CA, USA) in a Spectrometer SL 30 (Intertechnique, France).

Template RNA was degraded with 10% SDS, 0.5 M EDTA and 3 M NaOH during 30 min at 68 °C. Then, the reaction tube was placed at room temperature during 15 min. Finally, the labeled cDNA was neutralized (1 M Tris, pH 7.2, 2 N HCl) and denatured 5 min at 100 °C before the hybridization.

2.8. Hybridization and washing

Arrays were prehybridized in tubes (4 ml Screw Neck Vial, Alltech France sarl, Templemars, France) with 3 ml buffer (5× SSC, 5× Denhardt's, 0.5% SDS, 10% Dextran 50%, 100 μg/ml salmon sperm DNA previously denatured 5 min at 100 °C, 1μg dT80 in hybridization chamber (Qbiogene, Illkirch, France) at 60 °C, during 4–6 h.

Hybridization was performed in a volume of 700 μl during 72 h at 60 °C.

Arrays were washed twice for 15 min at 60 °C with 2 ml, 2× SSC, 1% SDS and then washed twice with 2 ml, 0.1× SSC, 0.5% SDS for 15 min. Arrays were allowed to air dry and were placed in a Fujifilm BAS Cassette 2025 (Raytest, Courbevoie, France) for 48 h.

2.9. Image acquisition

We used the phosphorimager Fujifilm BAS 5000 (Raytest, Courbevoie, France) for the detection of the radioactive signal, driven by Image Reader software BAS 5000 Version 1.12 (Raytest, Courbevoie, France). The signal was quantified by Image Gauge 4.0 software (Raytest, Courbevoie, France).

2.10. Real-time quantitative PCR

All cDNAs were prepared by reverse transcription of 2 μg of DNAse treated total RNA (young: $n = 3$ non-irradiated versus $n = 3$ UVA irradiated; adults: $n = 3$ non-irradiated versus $n = 3$ UVA irradiated; olders: $n = 3$ non-irradiated versus $n = 3$ UVA irradiated) in a 25 μl final volume as described above but without radiolabeled dCTP. Oligonucleotide primers were synthesized at Invitrogen (Invitrogen Life Technologies, Cergy-Pontoise, France). Primer design regarding to primer dimmer, self-priming formation and primer melting temperature was done with MacVector software (Accelrys, San Diego, USA). Specificities of the PCR amplification were documented with LightCycler melting curve analysis. Melting peaks obtained either from RT-product or from specific recombinant DNA were identical. Optimization of real-time quantitative PCR was carried out as described previously [21]. Intron spanning primer sets were designed for all genes (Table 3) excepted for mono-intronic gene GAS1. In this case, genomic DNA contamination was checked using RT minus samples.

The PCR was carried out with the LC Fast Start DNA Master SYBR Green kit (Roche Applied Science, Manheim, Germany) using 0.05 μl of cDNA (equivalent to 4 ng total RNA) in a 20 μl final volume, 4 mM $MgCl_2$ and 0.4 μM of each primer (final concentration). Quantitative PCR was performed using a Lightcycler (Roche Applied Science, Mannheim, Germany) for 45 cycles at 95 °C for 20 s, 57 °C (MMP1), 58 °C (GAPD, PPIA, MMP3), 59 °C (HMOX1, GAS1) or 60 °C (HPRT) for 5 s, and a final step of 10 s at 72 °C.

The threshold cycle (CT) value, was calculated from Lightcycler Software v.3.5 (Roche Applied Science, Mannheim, Germany) using the second derivative maximum method. Quantification was achieved according to the comparative threshold cycle method [20] using a pool of cDNA samples as calibrator [21]. Relative mRNA values were calculated with RealQuant software (Roche Applied Science, Mannheim, Germany). Normalization was performed by geometric averaging of three internal control genes, i.e. PPIA, GAPDH and HPRT [22] using RealQuant software (Roche Applied Science, Mannheim, Germany).

2.11. Data normalization and analysis

The data were treated to analyze gene expression. Firstly, we subtracted the signal background at each spot level and we identified visually each spot to exclude artifacts. Signals were normalized by dividing all signals with intensities above background by the median of all

Table 3
Primers used for real-time quantitative PCR

Gene name	5′–3′ forward primer	5′–3′ reverse primer	Amplicon (bp)
GAPD	GTATTGGGCGCCTGGTCACC	CGCTCCTGGAAGATGGTGATGG	202
GAS1	CAGAAAAGTCCCACTTACCGATTC	ACCAATTTAGGGGTTCCCTCTAAG	117
HMOX1	AGACTGCGTTCCTGCTCAACAT	GGGGCAGAATCTTGCACTTTGT	136
HPRT	CTCATGGACTAATTATGGACAGGAC	GCAGGTCAGCAAAGAATTTATAGCC	123
MMP1	GCTTACGAATTTGCCGACAGAG	TTCCTCAGAAAGAGCAGCATCG	162
MMP3	GGAGGAAAACCCACCTTACATACAG	CTTCCCAGACTTTCAGAGCTTTCTC	102
POLD1	AGGTGGTGTATGGTGACACTGACTC	GCAGGTATGGGAAGTAGACCTTCTC	159
PPIA	CATCTGCACTGCCAAGACTGAGTG	CTTCTTGCTGGTCTTGCCATTCC	127

signals. Both microarray and real time quantitative PCR results were analyzed by Unifactorial ANOVA to evaluate age effects on each gene expression profile. Bifactorial ANOVA was done to evaluate the effect of age and UVA exposure on gene expression. Statistical calculations were performed using Statview software.

3. Results

The results were obtained from a data set of 25 microarrays (young: $n = 5$ non-irradiated versus $n = 4$ UVA irradiated; adults: $n = 5$ non-irradiated versus $n = 4$ UVA irradiated; olders: $n = 4$ non-irradiated versus $n = 3$ UVA irradiated).

3.1. Transcript profile

Before analyzing age effects on gene expression modulation, we focused our attention on the basal transcript profile of the fibroblasts as a function of age. By this analysis, in the basal state, some transcripts were always detected, and others never, regardless of the age group considered.

Table 4
Transcripts always detected using the microarray

Gene name	Family	Intensity
Thioredoxin (TXN)	Antioxidant enzyme	+ +
Glutathione Peroxidase 1 (GPX1)	Antioxidant enzyme	+ +
Superoxyde Dismutase 1 (SOD 1)	Antioxidant enzyme	+
Superoxyde Dismutase 3 (SOD 3)	Antioxidant enzyme	+
Thioredoxin Reductase 1 (TXNRD1)	Antioxidant enzyme	+
Peroxydase (AOE 372)	Antioxidant enzyme	+
β-Actine (ACT)	House Keeping Gene	+ + +
Glutaraldehyde Phosphate Deshydrogenase (GAPDH)	House Keeping Gene	+ +
Ribosomal Protein L32 (RPL32)	House Keeping Gene	+ +
Xeroderma Pigmentosum group C (XPC)	DNA repair	+ + +
Ligase 4 (LIG4)	DNA repair	+
Polymerase D1 (POLD1)	DNA repair	+ + +
Matrix MetalloProteinase 2 (MMP2)	Extracellular matrix regulator	+ +
Matrix MetalloProteinase 3 (MMP3)	Extracellular matrix regulator	+ +
Mouse Double Minute 2 (MDM2)	Oncogene	+ +
Sar homology 2 domain containing (SHC1)	Oxidative stress response	+

The level of the transcript expression is represented by signs: + for transcript which expression value was inferior to 50% of the median value, + + when comprised between ± 50% of the median value and + + + when superior to 50% of the value of the median.

Table 5
Transcripts never detected using the microarray

Gene name	Family
Replication Protein 14 kDa (RPA 3)	DNA repair
Mut YH glycosylase (hMYH)	DNA repair
Radiation sensitive abnormal 52 (RAD52)	DNA repair
Excision Repair Cross Complementing 2 (ERCC2)	DNA repair
Excision Repair Cross Complementing 3 (ERCC3)	DNA repair
Excision Repair Cross Complementing 4 (ERCC4)	DNA repair
Ataxia Telangiectasia and Rad 3 related Frap related (ATR)	DNA repair
Breast Cancer 1 early onset (BRCA1)	DNA repair
Cockaine syndrome 1 (CKN1)	DNA repair
Thymine DNA glycosylase (TDG)	DNA repair
Radiation sensitive abnormal (RAD51)	DNA repair
8-oxoguanine DNA-glycosylase (OGG1)	DNA repair
Uracil-DNA glycosylase (UNG)	DNA repair
Thymine-DNA glycosylase (TDG)	DNA repair
General Transcrition Factor IIH peptide 2 (GTF2H2)	DNA repair
Transcription Protein 53 (p53)	DNA repair
Replication Protein A2 (RPA2)	DNA repair
Glutamate-Cysteine ligase, catalytic (GLCLC)	Antioxidant enzymes
Growth Arrest Specific 2 (Gas2)	Cell cycle regulator
c-fos transcription factor 4 (CROC4)	Oncogene
Telomerase reverse transcriptase (TERT)	Oncogene
Caspase 1 (CASP1)	Execution phase of apoptosis

The DNA repair family is the most represented with 17 transcripts never detected out of 37 spotted.

Seventeen transcripts related to antioxidant enzyme family ($n = 6$ out of 21 spotted), house keeping genes ($n = 3$ out of 5 spotted), DNA repair ($n = 3$ out of 32 spotted), extracellular matrix regulator ($n = 2$ out of 5 spotted), the oncogene MDM2 and the oxidative stress response gene SHC1, were always detected in the basal state and in all age groups. These results are presented in Table 4 where we specified the signal intensities. Transcripts of XPC and POLD1 were among the most expressed transcripts.

On the other hand, 22 transcripts were never detected in the human normal fibroblasts, in the basal state in all age groups. Among them, we noted that 17 were related to the DNA repair gene family (out of 37 spotted). Results are presented in Table 5.

3.2. Transcripts differentially expressed as a function of age in the basal state

Few transcripts were significantly modulated with age in cultured cells. Indeed, we noted that three transcripts varied significantly with ageing process (Fig. 1) demonstrating an epigenetic regulation. The transcript of MMP3, known as stromelysin 1 and related to the extracellular matrix regulator, increased with age. The expression level of MMP3 in children and adult groups was quite similar, whereas gene expression increased strongly in the older group ($p < 0.01$).

The transcript of thioredoxin (TXN) related to antioxidant enzymes family was more expressed in the adult group than in the older group ($p < 0.05$).

Expression of transcript of GAS1, a cell cycle regulator, strongly decreased with age and showed a significant difference between children and older groups ($p < 0.05$).

Even if expression of a few transcripts was significantly modulated as a function of age and showed some variability, some trends could be revealed by the analysis of hybridization signals. Indeed, it was difficult to obtain a statistical significance because of the variability between individuals and the low number of samples. We considered that transcripts showing modulation as a function of age with a p value comprised between 0.05 and 0.15 were the most interesting (see list of transcripts retained in Table 6).

The transcript of MMP1, related to an extracellular matrix regulator, increased with age, as also the transcript of MMP3. Furthermore, the transcripts of POLD1, LIG4 and Ku80 (XRCC5) related to DNA repair, increased with age.

On the contrary, transcript of GSS, related the family of antioxidant enzymes, tended to decrease with age as also the transcripts of MDM2, related to the family of oncogenes.

Some of these results were confirmed by real time quantitative PCR as we showed (Fig. 4): a decrease as a function of age for GAS1 transcript between children and olders ($p = 0.05$) and an increase with age for POLD1 transcript.

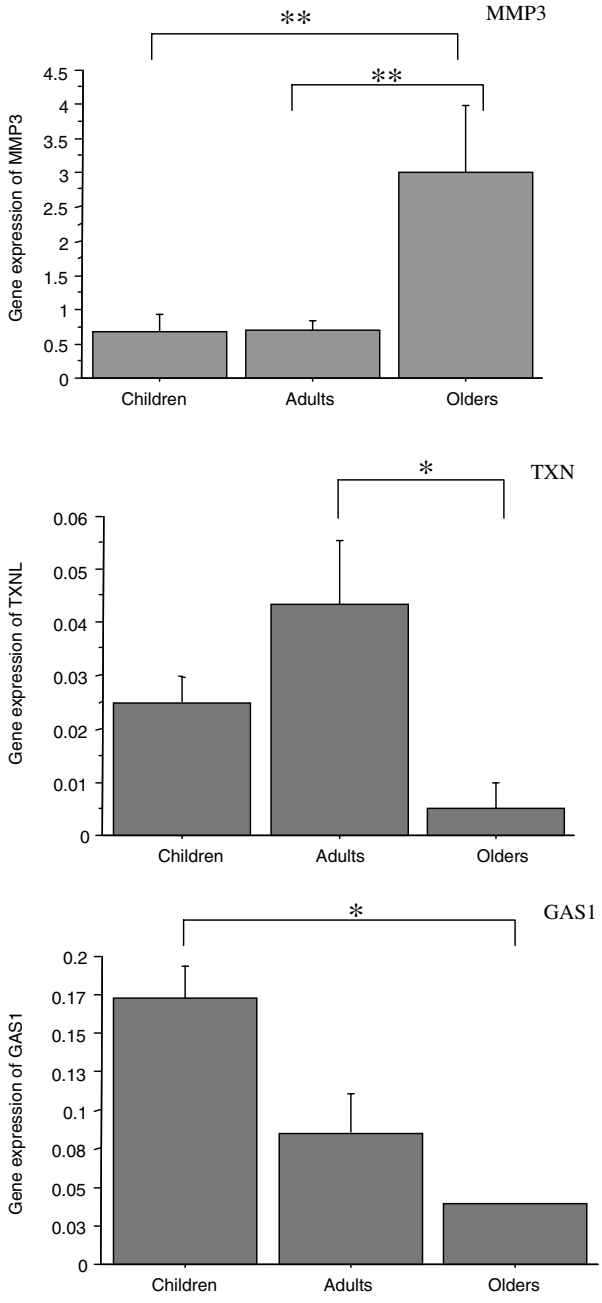

Fig. 1. Transcripts differentially expressed as a function of age at the basal state. The graphs represented gene expression normalized (with the median of gene which value is superior to the background value) as a function of age. Results are represented by the mean ± SD of 14 analyzed microarrays (children, $n = 5$; adults, $n = 5$; olders, $n = 4$) with $^{**}p < 0.01$ and $^{*}p < 0.05$.

3.3. Response to 5 J/cm^2 UVA exposure after 6 hours of post-treatment incubation

Human fibroblasts in primary cultures, from different age groups, were UVA irradiated at 5 J/cm^2. Then, the

Table 6
Transcripts which expression is modulated as a function of age, at the basal state

Gene name	Family
Gene expression increasing with age	
Matrix Metalloproteinase 1 (MMP1)	Extracellular matrix regulation
Polymerase Delta subunit 1 (POLD1)	DNA repair
Ligase 4 (LIG4)	DNA repair
X-ray Cross Complementing defective repair in Chinese hamster cells 1 (XRCC5)	DNA repair
Gene expression decreasing with age	
Glutathione Synthetase (GSS)	Antioxidant enzyme
Mouse Double Minute 2 (MDM2)	Oncogene

Transcripts were retained when p value was comprised between 0.05 and 0.15.

cells were allowed to repair for 6 h and total RNAs were isolated. The gene expression profiles were studied 6 h after UVA irradiation.

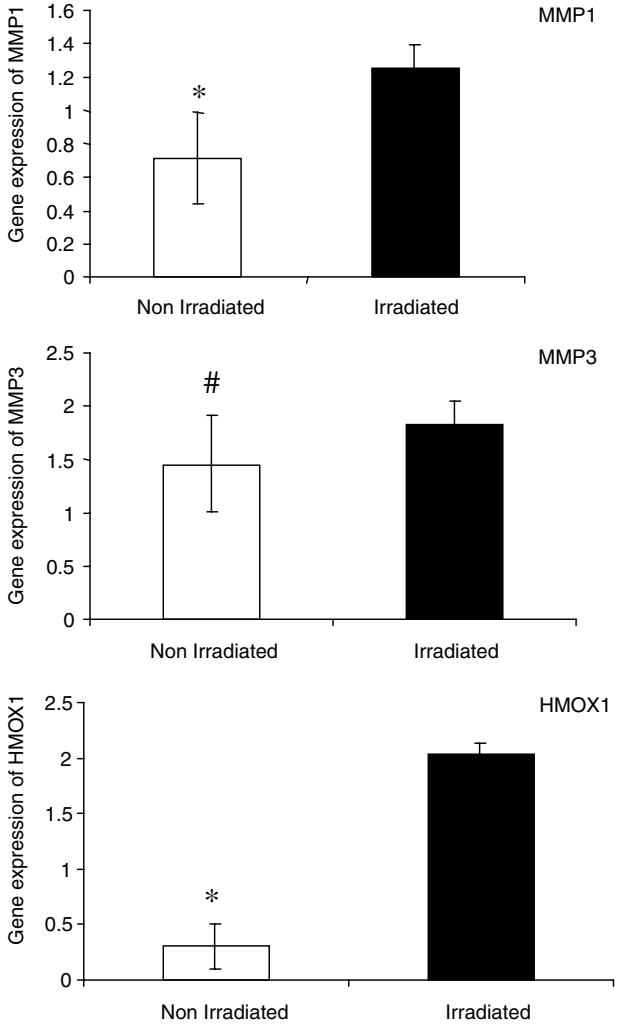

Fig. 2. Response to 5 J/cm^2 UVA irradiation 6 h after the stress. Results are represented by the means ± SD of 25 analyzed microarrays (children, $n = 5$ non-irradiated and $n = 4$ irradiated; adults, $n = 5$ non-irradiated and $n = 4$ irradiated; olders, $n = 4$ non-irradiated and $n = 3$ irradiated); $^{*}p < 0.05$ irradiated cells versus non-irradiated cells and $^{\#}p < 0.1$ irradiated cells versus non-irradiated cells.

In all age groups, we observed that three genes were significantly induced by UVA irradiation: MMP1 ($p < 0.05$), HMOX1 ($p < 0.05$) related to antioxidant defences which showed the strongest induction and MMP3 ($p < 0.1$) (see Fig. 2).

These results were confirmed by real time quantitative PCR (Fig. 4).

Two transcripts were modulated by both, UVA radiation and age: POLD1 and APEX (Fig. 3).

Indeed, UVA irradiation significantly ($p < 0.05$) repressed POLD1 expression in the adult group, whereas

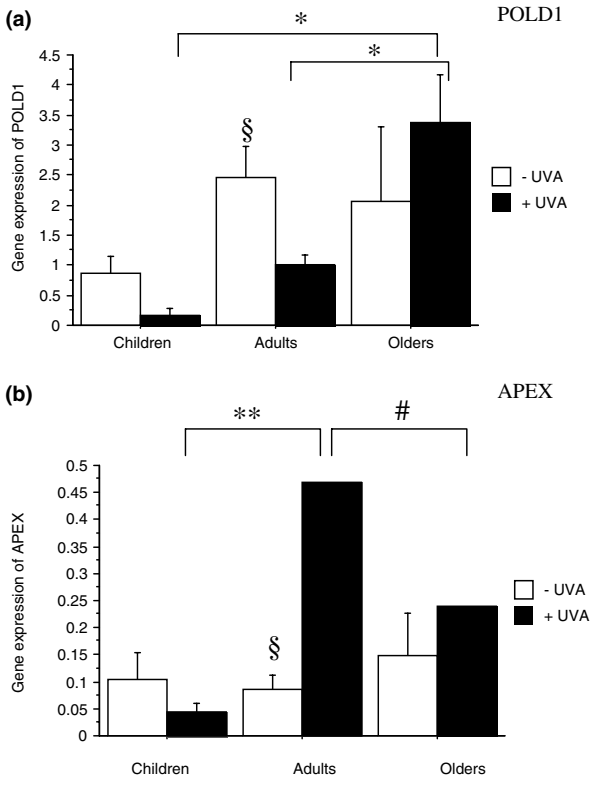

Fig. 3. Modulation of transcript expression, as a function of age and 6 h after a 5 J/cm^2 UVA irradiation. Results are represented by the means ± SD of 25 analyzed microarrays (children, $n = 5$ non-irradiated and $n = 4$ irradiated; adults, $n = 5$ non-irradiated and $n = 4$ irradiated; olders, $n = 4$ non-irradiated and $n = 3$ irradiated); $^{**}p < 0.01$, $^{*}p < 05$; $^{\#}p <$ 1and $^{\S}p < 0.05$ irradiated cells versus non-irradiated cells.

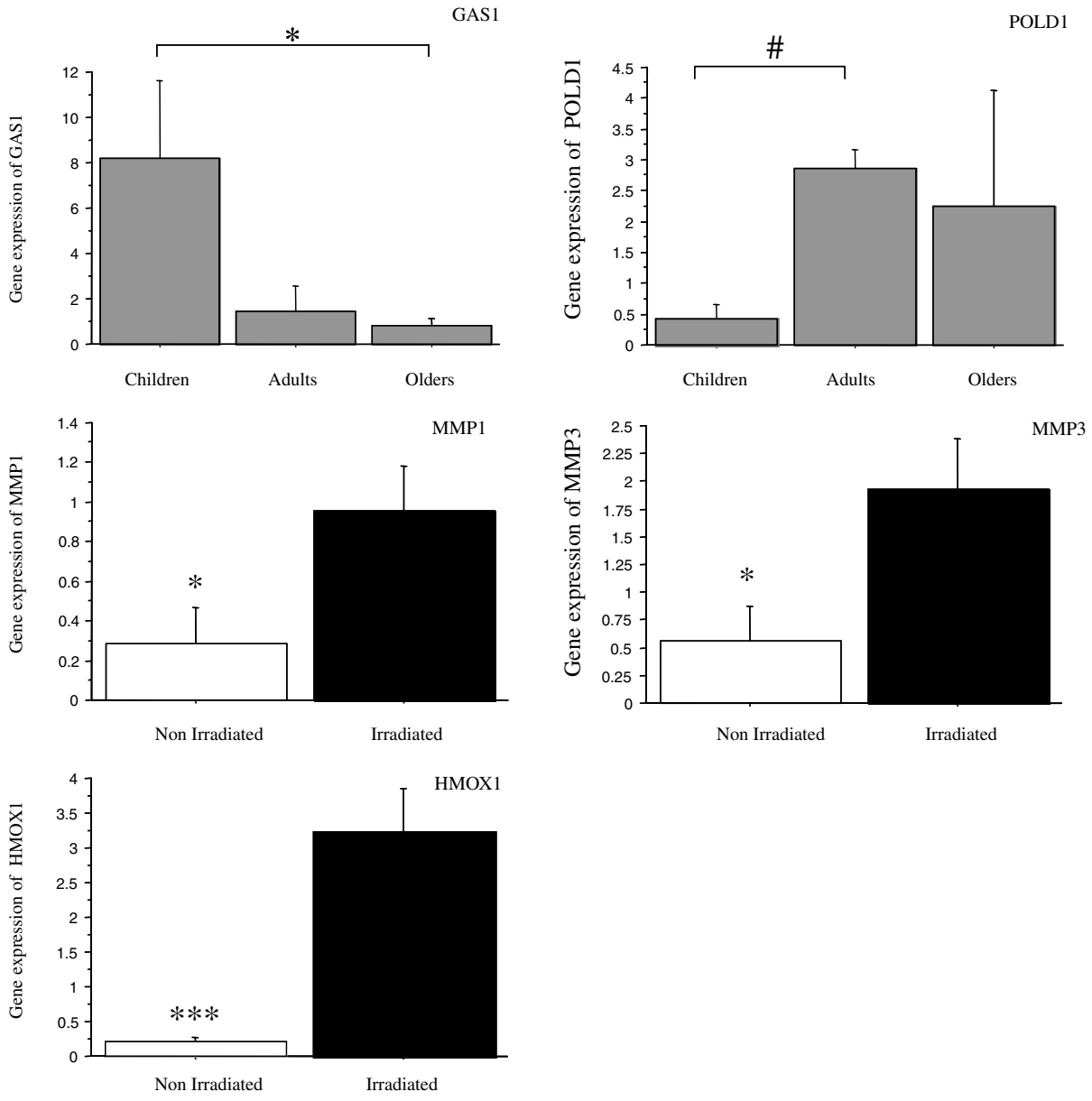

Fig. 4. Expression levels of GAS1, POLD1 at the basal state and MMP1, MMP3 and HMOX1 6 h after a 5 J/cm^2 UVA irradiation, using real-time quantitative PCR. Results are represented by relative unit values normalized by geometric averaging of three internal control genes: PPIA, GAPDH and HPRT. Data are means ± SD obtained from children: $n = 3$ non-irradiated and $n = 3$ irradiated, adults: $n = 3$ non-irradiated and $n = 3$ irradiated, olders: $n = 3$ non-irradiated and $n = 3$ irradiated with $^{***}p < 0.001$, $^{*}p < 0.05$ and $^{\#}p < 0.1$.

UVA effect on POLD1 expression was not significant in the children and the older groups (Fig. 3(a)). POLD1 expression after UVA irradiation was significantly more important in the older group than in the children ($p < 0.05$) and in the adult ($p < 0.05$) groups. This result confirms the tendency noted for the basal state, in which POLD1 expression increased with age (Table 6). However, this different expression of POLD1 after UVA irradiation between the three age groups was not found by real time quantitative PCR. This discrepancy could be due to the too small number of samples.

With regard to the APEX expression in irradiated versus unirradiated samples of each age group, only cells belonging to the adult group showed increased expression ($p < 0.05$). In the children and the older groups (Fig. 3(b)), the level of APEX transcript was comparable between non-irradiated and irradiated cells. Indeed, gene expression of APEX was more important in the adult group than in the children group ($p < 0.01$) and higher than in the older group ($p < 0.1$) (Fig. 3(b)).

Furthermore, in this analysis an interesting trend was observed for the transcript of ligase 4. After UVA irra-

diation, the induction was more important in the older group than in the adult group ($p < 0.1$) or children group ($p = 0.1$).

4. Discussion

The aim of this study was to examine the gene expression associated with ageing in human primary fibroblasts and the influence of ageing on cellular responses after 5 J/cm^2 of UVA irradiation. For this purpose, we used expressed sequence tags (ESTs), representing gene specific sequences of cDNA, spotted on nylon arrays to explore the transcriptional changes and to analyze the responses to the ageing process and UVA induced oxidative stress. We created a specific array to focus our attention on the analysis of gene expression related to cellular defence systems. Some of the results obtained by our microarray were confirmed by real time quantitative PCR.

We chose to expose the fibroblasts to a weak UVA dose of 5 J/cm^2 and thus to focus our analysis on genes induced by doses frequently encountered in daily life. Moreover, it was previously shown that a dose of 5 J/cm^2 of UVA induces a response in fibroblasts that did not involve cytotoxicity [23,24]. Moreover, we were particularly interested in genes responding rapidly that is to say within a few hours after exposure. Previous studies have shown that between 4 and 6 h after the stress several genes were induced, particularly those with reparative, protective or apoptotic functions [25,26]. Hence, we recolted fibroblasts 6 h after UVA irradiation.

We needed at least 5 μg of total RNA for hybridization experiment. This quantity was difficult to obtain as we chose not to propagate fibroblasts beyond the tenth culture passage in order to prevent in vitro ageing. Although we were limited for RNA quantity, we chose to do biological replicates (array hybridized with RNA from independent cell cultures) rather than technical replicates (different arrays hybridized with RNA derived from one cell culture) by pooling samples from the same age group. Pooling is subjected to controversy because it hides individual variability [27]. Nevertheless, when the amount of RNA was sufficient we also performed technical replicates.

From the analysis of hybridization signal in our array, we edited a transcriptional profiles for the treated and untreated fibroblasts.

We noted that only 16 transcripts out of 81 spotted were always detected. The majority was related to antioxidant enzymes (6 out of 17 spotted). MMP2 and MMP3, involved in the regulation of the extracellular matrix and playing an essential roles in fibroblast metabolism, were also always detected.

In the basal state, the transcripts of XPC and polymerase delta 1, catalytic subunit 125 kDa (POLD1), related to DNA repair, presented the strongest signal. XPC is involved in the initial step of NER process, i.e. in the recognition of DNA lesions. In the basal state, POLD1, XRCC5 (Ku80) and LIG4 tended to increase in the older group. Interestingly, age dependent and UVA dependent induction of genes was observed for the genes POLD1 and APEX. POLD is a heterodimer with subunits of 125 and 50 kDa. The 125-kDa subunit possesses proof reading activity [28] and is involved in replication and repair of DNA [29]. The 50-kDa subunit (POLD2) can interact with C-terminal region of the Werner protein (WRN). Werner's syndrome (WS) is caused by mutations within a single gene encoding the protein WRN. This belongs to the family of human RecQ helicase [30]. WS is a human genetic disease with many features of premature ageing. Moreover, a cellular WRN complex has been identified, composed of WRN, Ku70/Ku80 and poly (ADP-ribose)polymerase-1 (PARP) [31]. The Ku heterodimer (Ku70 and Ku80) binds with high affinity to DNA ends of double-strand breaks (DSB), thus Ku localized to DSB. Ku is required for the regulation of telomere length and the repair of DSB by non homologous end-joining (NHEJ) [32]. Interestingly, Ku and WS have been found to be involved in regulatory mechanisms of ageing. The fact that XRCC5 (Ku80) and POLD1 transcripts tended to increase with age could reflect an increase in the need of regulator proteins counteracting the ageing effects. Personal as yet unpublished data show an age-dependent increase in DNA damage as measured by the comet assay and decrease in DNA repair. We cannot exclude that translation to proteins could be less efficient and/or that half life of proteins could be reduced, with age. Furthermore, it has been suggested that oxidative DNA damage accumulates with age [33]. Thus, changes in transcript levels should be related to cellular protection against DNA damage, ageing and cancer.

A study by Li et al. [34] showed that POLD1 gene is repressed by wild-type p53 in response to DNA damage. Interestingly, the transcript of p53 is among the never detected ones. The protein p53 [35] has multifunctional properties related to cell cycle arrest, drug detoxification, apoptosis, signal transduction and others. It can induce cell cycle arrest or apoptosis to allow repairing cell damage created under stress. Nevertheless, neither transcript of p53 nor transcript of caspases where detected, at the basal state and 6 hours after the 5 J/cm^2 UVA irradiation (Table 5). It has been reported in several studies that fibroblasts were resistant to apoptosis. Indeed, primary normal fibroblasts cultured in vitro were resistant to apoptosis after IR exposure [36]. Another study showed that primary normal fibroblasts cell lines did not respond to a 100 Gy irradiation, 24 h after the stress [37]. However Fluck et al. [38] demonstrated that primary normal fibroblasts growing in vitro in a three dimensional contractile collagen matrix were more

susceptible to apoptosis than fibroblasts cultured in monolayer.

We noted that MMP3 and MMP1 increased with age at the basal state (Fig. 1 and Table 6 respectively) and after irradiation (Fig. 2). MMP3, also known as stromelysin 1, degrades fibronectin, laminin, collagens III, IV (principal component of basal membranes) IX and X. MMP1, also named interstitial collagenase, is involved in the breakdown of collagens I, II and III. It has been demonstrated that mRNA coding for MMP1 was increased during ageing, using fetal and adult fibroblasts [39]. MMP1 was increased in skin exposed to sun [40], after 1 MED irradiation [41] and in skin of smokers [42]. Interestingly, a microarray gene expression analysis by Shelton et al. [17] demonstrated that MMPs were increased during replicative senescence in fibroblasts. It appears that the same trend is observed between intrinsic ageing and photoaging. It can be concluded that expression of matrix-degrading proteases in fibroblasts is a consistent trend of ageing and a consequence of the oxidative stress that leads to structural and functional changes observed in the skin during the intrinsic ageing (see Table 7).

A literature search revealed that the status of the thioredoxin system (TXN) during ageing has not been studied extensively and that the existing data are often discordant. At the basal state, we observed that transcript of TXN decreased between adult and older groups (Fig. 1; $p < 0.05$) and that transcript of glutathione synthetase (GSS, implicated in the regeneration of glutathione, essential element in antioxidant defences and redox state in the cell) tended to decrease between adult and older groups (Table 6; $p < 0.15$). TXN and GSH system are involved in a variety of redox dependent pathways [43]. TXN and GSH are implicated in cell defences against oxidative damage on proteins and in maintaining the redox status in the cell. In our work, except for TXN and GSS, we did not find any difference in antioxidant enzyme activities, at the basal state and after irradiation. Some studies pointed out the activities or the levels of transcripts of antioxidant enzymes during ageing and their conclusions were different. Nevertheless, some works pointed out variations in antioxidant enzymes activities with ageing, particularly of catalase, which decreased in naturally aged human skin in vivo and in photoaged skin [44]. Shindo et al. [45] who also used a human skin fibroblast model, found a slight increase in GPX activity with age and observed a significant loss of catalase activity. On the other hand, Keogh et al. [46] observed no difference with age of glutathione peroxidase (GPX) and catalase activities when comparing dermal fibroblasts of young and older donors. The modification of the antioxidant status during ageing process is not clear and probably undergoes a complex regulation during the process. An animal model [47] showed that TXN cytoplasmic protein levels decreased with age. It has been demonstrated that TXN was quickly translocated into the nucleus upon UV irradiation [48]. In the nucleus, TXN can interact with Ref-1 gene product [49], known as APE-1 and APEX that repairs DNA damage and acts as a DNA binding protein on genes such as transcription factors AP-1 and NF-κB. The complex TXN-Ref-1 is able to control the redox status by regulating transcription factors implicated in cell response to oxidative stress. In this study, we demonstrated that

Table 7
This table summarises the main results obtained by microarray at the basal state and after 5 J/cm^2 UVA irradiation, with $^*p < 0.05$ and $^{**}p < 0.01$

Genes	Expression profiles			See details in
	Age effect on gene expression at the basal state	UVA effect on gene expression	Effects of UVA + age on gene expression	
MMP3	↑with age*	↑		Fig. 1/Fig. 2
TXN	More important in the adult group*			Fig. 1
GAS1	↓with age*			Fig. 1
MMP1	↑with age*	↑*		Table 6/Fig. 2
POLD1	More important in the adult and older groups than the children group	Decreased of POLD1 in the adult group*	More important in the older group than in the adult and the children group*	Table 6/Fig. 3
LIG4	↑with age		↑with age	Table 6
XRCC5	↑with age			Table 6
GSS	↓with age			Table 6
MDM2	↓with age			Table 6
HMOX1		↑*		Fig. 2
APEX		Strong induction in the adult group*	More important in the adult group than in the children and the older groups**	Fig. 3

When no statistical annotation appears, the results obtained had a p value comprised between 0.05 and 0.15.

the transcript of APEX was induced 6 h after UVA irradiation (Fig. 3) and that the induction was only significant in the adult group. Transcript levels of TXN and APEX evolved with age. Thus, cellular responses following oxidative stress are likely to change with age. Indeed, it has been proposed that ageing could result from a long-term cellular redox imbalance [50,51].

Interestingly, we observed that in the basal state, the transcript of GAS1 decreased with age (children versus older groups, $p < 0.05$, Fig. 1). Studies on GAS1 are recent. The gene encodes for a glycosyl-phosphatidylinositol (GPI) anchor protein associated with the plasma membrane. It can induce growth arrest via a p53-dependent signaling pathway [52,53]. It is overexposed in proliferating fibroblasts in response to stimuli driving the cells into G0 phase. It is involved in growth arrest and in suppression of tumorigenicity of human cells [54,55]. The study of Shelton et al. [17] on replicative senescence in fibroblasts revealed that GAS1 was overexposed during the in vitro ageing. Our current data on GAS1 suggest that entry into quiescent phase of the cell cycle is prevented. As a consequence, cells would be more susceptible to proliferate with DNA damage. The changes in GAS 1 expression are associated with a decrease of MDM2. This gene is also involved in cell cycle and apoptosis by acting on p53. This relates to the observation that abnormalities in the cell cycle during ageing may perturb DNA repair and favor carcinogenesis [56].

UVA radiation induces HMOX that protects the cell against heme liberation by oxidants [57]. In accord with this, we show here that the transcript of heme oxygenase-1 (HMOX1) is induced by a 5 J/cm^2 UVA irradiation. The last result confirms the validity of our approach using microarrays.

In line with earlier reports [58], the transcript of TERT was never detected in our conditions. The activity of TERT is associated with the control of cell divisions and the lifespan of human cells [59]. Telomeres are implicated in the stabilization of the ends of eukaryotic chromosomes.

In conclusion, the present study documents age and UVA related changes in transcription profiles in normal skin fibroblasts. We studied the effects of age and UVA in relation to DNA repair activity, cell cycle and to extracellular matrix biology. Indeed, our report shows that MMPs transcripts increase with age and UVA exposure, thus contribution to the ageing process by modeling the ECM. We also show that transcripts of the DNA repair genes POLD1, LIG4, Ku80 increase with age, suggesting an age-dependant need for these cell defence systems. We also demonstrate that the genes TXN, GSS and APEX which are important genes contributing to the regulation of redox status of cells, are induced to a lesser extent in the older group and after UVA-induced oxidative stress.

5. Abbreviations

AP-1	activator protein 1
BER	base excision repair
DSB	double strand break
ECM	extracellular matrix
EST	expressed sequence tag
MMR	mismatch repair
NER	nucleotide excision repair
ROS	reactive oxygen species
WS	Werner's syndrome

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