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Gene Expression Profiles in Radiation Workers Occupationally Exposed to Ionizing Radiation

Ana Lucia FACHIN[1], Stephano Spano MELLO[1], Paula SANDRIN-GARCIA[1], Cristina Moraes JUNTA[1], Thomaz GHILARDI-NETTO[4], Eduardo Antonio DONADI[2], Geraldo Aleixo da Silva PASSOS[1,3] and Elza Tiemi SAKAMOTO-HOJO[1,5]*

Low radiation doses/Ionizing radiation/Radiation workers/Occupational exposure/Gene expression profiles.

Ionizing radiation (IR) imposes risks to human health and the environment. IR at low doses and low dose rates has the potency to initiate carcinogenesis. Genotoxic environmental agents such as IR trigger a cascade of signal transduction pathways for cellular protection. In this study, using cDNA microarray technique, we monitored the gene expression profiles in lymphocytes derived from radiation-exposed individuals (radiation workers). Physical dosimetry records on these patients indicated that the absorbed dose ranged from 0.696 to 39.088 mSv. Gene expression analysis revealed statistically significant transcriptional changes in a total of 78 genes (21 up-regulated and 57 down-regulated) involved in several biological processes such as ubiquitin cycle (*UHRF2 and PIAS1*), DNA repair (*LIG3, XPA, ERCC5, RAD52, DCLRE1C*), cell cycle regulation/proliferation (*RHOA, CABLES2, TGFB2, IL16*), and stress response (*GSTP1, PPP2R5A, DUSP22*). Some of the genes that showed altered expression profiles in this study can be used as biomarkers for monitoring the chronic low level exposure in humans. Additionally, alterations in gene expression patterns observed in chronically exposed radiation workers reinforces the need for defining the effective radiation dose that causes immediate genetic damage as well as the long-term effects on genomic instability, including cancer.

INTRODUCTION

Ionizing radiation (IR) is a ubiquitous environmental agent whose effects on DNA-damaging are well established. A direct interaction of IR with cellular targets produces a variety of primary lesions: single-strand breaks (SSBs), alkali-labile sites, double-strand breaks (DSBs), DNA–DNA and DNA–protein cross-links and damage to purine and pyrimidine bases.[1)] Effects of IR at chronic low doses and dose rates have been considered to be mutagenic and carcinogenic in humans. Cytogenetic studies demonstrated that even low levels of chronic radiation exposure increase the frequencies of chromosomal aberrations.[2–6)] Therefore, health and welfare of human population occupationally exposed to chronic low dose radiation are of great concern. Although it is possible to estimate the absorbed radiation dose for occupationally exposed-individuals, the extent of long-term health consequences is difficult to assess. Therefore, development of new strategies is critical for the reliable estimation and assessment of radiation exposure in humans.

Cellular responses to IR involve a complex network of signal transduction pathways. In the past decade, cellular activities involving the concerted action of DNA repair and cell cycle checkpoint have been clearly elucidated.[7–9)] Several gene expression studies demonstrated an up-regulation of genes involved in the processes of signal transduction, cell cycle control, DNA repair and apoptosis after IR exposure in different mammalian cell types.[10–17)] These studies revealed considerable qualitative and quantitative variations in the expression profiles of genes. Variations are presumably due to different cell systems and radiation doses employed in those studies. Very few genes (*GADD45*, and

*Corresponding author: Phone: +55-16-3602-3082,
Fax: +55-16-3633-0069,
E-mail: etshojo@usp.br
[1]Departamento de Genética, Faculdade de Medicina de Ribeirão Preto-USP, Av Bandeirantes 3900, 14049-900 Ribeirão Preto, SP, Brasil; [2]Departamento de Clínica Médica – FMRP-USP; [3]Faculdade de Odontologia de Ribeirão Preto-USP; [4]Centro de Ciências das Imagens e Física Médica, HCFMRP-USP, [5]Departamento de Biologia, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto-USP, Universidade de São Paulo, Ribeirão Preto, SP, Brazil.
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CDKN1A) have been found to be consistently up-regulated by IR, and interestingly, genes involved in nucleotide excision repair pathway (XPA and XPC) have been characterized as IR-responsive.[18)]

Although many of the existing studies demonstrated altered expression of genes after IR in a variety o cell systems at relatively high radiation doses (1–4 Gy), there still remains considerable uncertainty about the impact of chronic low dose of IR on gene expression profiles in human populations. With this objective, the present study was performed on gene expression changes due to occupational exposure to low IR doses in peripheral blood lymphocytes taken from radiation workers. Our study demonstrates the altered expression of at least 78 genes in these workers. Some of these genes, after validation in large population samples, can be used for biomarkers for the assessment of chronic low dose radiation exposure in humans.

MATERIALS AND METHODS

Subjects and sample collection

Peripheral blood samples were taken from healthy donors. Out of the 23 samples, 14 of them had the record of occupational exposure to radiation while the remaining 9 samples were from unexposed human individuals. All the persons completed a standardized questionnaire that included information regarding personal data (age and health status), time of employment (involving occupational exposure to IR up to the sampling time), non-occupational exposure to potential mutagenic hazards, life style (smoking, alcohol consumption), viral diseases, radiodiagnostic examinations, and drug therapies. Only healthy individuals were included in the study, those who had taken more than three radiodiagnostic examinations and submitted to known genotoxic/clastogenic agents during the period of employment were excluded from the study. Further, radiodiagnostic examinations in all cases included only simple radiography procedures, and none of subjects was exposed to more than 0.5 mSv of medical radiation. Care was taken to ensure that all the included individuals in this study were not alcohol consumers.

Age of control group (n = 9, 3M and 6F) showed the mean (± SD) age of 37.2 ± 9.42, while the exposed group (n = 14, 5M and 9F) showed 41.14 ± 6.77 years (Table 1). The radiation workers (physicians, nurses and radiological technicians) were employees of several sectors (hemo-dynamics, nuclear medicine and radio-diagnosis) in a local Hospital (Ribeirao Preto, SP, Brazil). Their mean (± SD) period of occupational exposure was 9.32 ± 5.97 years. Since the occupationally exposed workers wore personal dosimeter film badges, physical dosimeter records were available during their work activities for the entire employment period. Physical dosimeter records indicated that the total accumulated radiation doses in these individuals varied from 0.696 to 39.088 mSv, mean (± SD) = 7.67 ± 10.16 (Table 1). Informed consents were obtained from all the sampled individuals only after the approval of the term and research project by the local Ethics Committee (Faculty of Medicine, Ribeirao Preto-USP, SP, Brazil).

Total RNA extraction

Total peripheral blood mononuclear cells were isolated by gradient density using Ficoll-Hypaque (Sigma, Saint Louis, MO), following RNA extraction with the Trizol® reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The purity of RNA samples was evaluated by denaturing agarose gel electrophoresis under standard conditions, and Northern-blot analysis was performed using an oligonucleotide probe specific for the 28S rRNA fraction (data not shown). To remove the contaminating DNA, RNA samples used in cDNA microarrays were treated with the Deoxyribonuclease I (Amplification Grade kit, Invitrogen), according to the manufacturer's instructions.

cDNA microarray method

Experiments with occupationally exposed individuals were carried out using a glass slide microarrays containing 4500 clones of cDNA probe (in duplicates) from the human IMAGE Consortium cDNA library [http://image.llnl.gov/image/; kindly provided by Dr. Catherine Nguyen (INSERM-CNRS, Marseille, France)], and prepared according to the protocol described by Hegde *et al.*[19)]

Microarrays were spotted onto glass slides (Corning) by using a Generation III Array Spotter Amersham-Molecular Dynamics according to the manufacturer's instructions. Each cDNA sample was spotted twice in the slide (duplicate spots). The cDNA complex probes were prepared using the CyScribe Post Labelling Kit (Amersham Biosciences, England) in two steps: I. 10 μg of total RNA was transcribed into cDNA with the addition of a chemically reactive nucleotide analog (aminoallyl-dUTP); II. the synthesized cDNA was "post labeled" with the reactive forms of Cy3- or Cy5-NHS esters, which bind to the modified nucleotides. Additional details about purification steps and reactivity controls are supplied in the manufacturer's manual. A pooled reference sample was applied in the present work, so that every microarray hybridization was performed with the identical reference sample (reference pool). The pooled reference sample was labeled with Cy3, while the experimental samples (control or treated) were labeled with Cy5. This procedure facilitates normalization of data to the pooled reference sample. The reference sample was constructed using RNA extracted from three human cell lines: U343 MG-a (glioma cells), HeLa (epithelial cervical carcinoma) and Jurkat (T-cell leukemic cells). RNA from these cell lines was extracted, pooled in equal ratios, and stored at –80°C until use.

Hybridizations were carried out at 42°C for 15 hours using an automatic system (Automatic Slide Processor, Amersham Biosciences, England) and signals were immedi-

Table 1. Characterization of medical personnel occupationally exposed to ionizing radiation and control individuals.

Subjects	Age (years), Sex	Time of employment (years)	Type of Radiation	Smoking habit	Dose (mSv)	Radiation Service
E1	51, F	17	X, γ, β-rays	No	5.027	M, H
E2	30, M	6	β-rays	No	1.539	M
E3	42, F	4	X-rays	No	0.696	H
E4	48, M	7	X-rays	No	4.66	H
E5	31, F	3	B, γ-rays	No	2.473	M
E6	38, M	5	X- rays	No	3.641	H
E7	52, F	13	X, γ, β-rays	No	5.596	M, H
E8	45, F	16	X, γ, β-rays	No	1.895	M, H
E9	38, M	7	X-rays	No	18.226	H
E10	43, F	15	X, γ-rays	Yes	11.686	H
E11	43, F	17	X- rays	No	4.19	R
E12	34, M	1.5	X-rays	No	39.088	H
E13	38, F	3	Γ, β-rays	Yes	1.895	M
E14	43, F	16	X, γ-rays	No	6.847	H
C1	48, F	–	–	Yes	–	–
C2	31, F	–	–	No	–	–
C3	28, M	–	–	No	–	–
C4	48, F	–	–	No	–	–
C5	43, F	–	–	Yes	–	–
C6	22, M	–	–	No	–	–
C7	32, M	–	–	No	–	–
C8	45, F	–	–	No	–	–
C9	38, F	–	–	No	–	–

Dose; M: Nuclear Medicine; H: Hemo-dynamics; R: Radiodiagnostic;
Gender: F (female); M (male).

ately captured after the final wash procedure, using a Generation III laser scanner (Amersham Biosciences, England).

Gene expression analysis by the cDNA microarray method

The image quantification was performed using the Spot software, (http://spot.cmis.csiro.au/spot/, CSIRO, Australia). Filtering, normalization and the data analysis were done using the R statistical environment[20] in addition to Limma,[21] Bioconductor,[20] Aroma[22] and KTH.[23] The background to each feature was subtracted from the foreground value. Furthermore, the spots were evaluated by their circularity and calculations on the median *versus* mean deviation, so that those presenting irregular circularity, or with large differences between mean and median values, were considered unreliable. The raw data (red – R and green – G) was transformed into MA format before normalization, where $M = \log2(R/G)$ and $A = 1/2 \times \log2(R \times G)$. These procedures were followed by the application of the Print-tip Lowess normalization for each slide. Moreover, we also carried out a normalization between slides, re-scaling the M values distribution using the Median Absolute Deviation value (MAD).[24]

Following the normalization procedure, microarray data were exported to tab-delimited tables in MEV format and analyzed in MEV (v. 3.1) software, a versatile microarray data analysis tool, which incorporates clustering algorithms, visualization, classification, and statistical analysis.[25]

Detection of changes in gene expression profiles caused by chronic low doses of radiation in occupationally exposed individuals posed a technical problem due to inter individual variability. To achieve a confident list of modulated genes,

Table 2. Modulated genes observed for lymphocytes from radiation workers occupationally exposed to ionizing radiation, as selected by the SAM method, FDR < 5.36%.

Genes	Clone.ID	Fold-change	q-value	Function
SLC45A4	40040	1.79	3.15	Transport
ADIPOR2	143067	1.29	3.15	Fatty acid oxidation
CCL4	259552	2.51	4.61	Cell adhesion
HBB	142422	2.47	4.61	Hemoglobin/oxygen transport
HBA1	142947	2.14	4.61	Hemoglobin/oxygen transport
PACRG	39202	1.88	4.61	Unknown
–	38975	1.81	4.61	Unknown
LIG3	2326929	1.77	4.61	DNa repair
R3HDM1	21627	1.67	4.61	Unknown
C9orf82	39076	1.53	4.61	Unknown
–	34966	1.52	4.61	Unknown
PDCD6	40136	1.51	4.61	Apoptosis
HILS1	40107	1.49	4.61	Chromatin remodeling
PPP2R5A	41356	1.46	4.61	Stress response
LRRK1	39136	1.43	4.61	Signal transduction
A2BP1	34949	1.39	4.61	RNA splicing
XPA	5214347	1.37	4.61	DNA repair
–	35105	1.24	4.61	Unknown
–	25507	1.23	4.61	Unknown
UHRF2	23042	1.18	4.61	Ubiquitin cycle
TANC2	139349	1.16	4.61	Unknown
ACAT2	36393	–1.16	0.00	Lipid metabolism
VAMP1	32021	–1.50	0.00	Vesicle-mediated transport
ERCC5	1308118	–1.77	0.00	DNA repair
GSTP1	136235	–1.77	0.00	Response to stress
HS6ST3	32003	–1.80	0.00	Transferase activity
HNRPF	136606	–1.22	1.83	RNA processing
TMEM138	144902	–1.40	1.83	Unknown
ACP6	265388	–1.51	1.83	Lipid metabolism
PRAGMIN	142532	–1.52	1.83	Protein amino acid phosphorylation
KIAA0415	21454	–1.53	1.83	Unknown
SLC25A25	32719	–1.56	1.83	Transport
C2orf44	136433	–1.64	1.83	Unknown
TRIM22	136965	–1.39	3.67	Zinc binding domain
ROBO1	140011	–1.50	3.67	Cell adhesion/differentiation
IL16	31839	–1.58	3.67	Cell cycle control
MAP2K1	33826	–1.70	3.67	Protein amino acid phosphorylation/MAPK
KDELC2	136126	–1.35	3.83	Unknown
–	25882	–1.40	3.83	Unknown

Continued.

Table 2. Continued.

Genes	Clone.ID	Fold-change	q-value	Function
EML4	24859	–1.40	3.83	Mitosis
KBTBD6	22144	–1.48	3.83	Unknown
–	144846	–1.48	3.83	Unknown
–	25487	–1.15	4.59	Unknown
GPR83	36369	–1.33	5.00	Signal transduction
INPP4B	165857	–1.14	5.36	Signal transduction
DCLRE1C	139197	–1.18	5.36	DNA repair/recombination
TGFB2	36472	–1.19	5.36	Cell proliferation
KIF1A	221828	–1.20	5.36	Transport
–	134056	–1.21	5.36	Unknown
DLX6	135807	–1.21	5.36	Transcription factor/neurogenesis
LOC286440	23039	–1.21	5.36	Unknown
LOC729970	36906	–1.22	5.36	Unknown
–	136571	–1.23	5.36	Unknown
REPS1	37392	–1.23	5.36	Unknown
ABCA7	182933	–1.26	5.36	Transport
–	181796	–1.27	5.36	Unknown
STX8	139993	–1.27	5.36	Transport
FLJ32549	21696	–1.28	5.36	Unknown
PIGA	35971	–1.29	5.36	Preassembly of GPI anchor in ER membrane
PIAS1	32565	–1.30	5.36	Ubiquitin cycle/cell communication/signaling
TOM1	141718	–1.31	5.36	Endocytosis/intra-golgi transport
KIAA0853	137271	–1.33	5.36	Unknown
DUSP22	182999	–1.35	5.36	Stress response
PFDN1	134673	–1.36	5.36	Protein folding
NPEPPS	132012	–1.36	5.36	Photolytic events regulating the cell cycle
DHX40	263883	–1.39	5.36	RNA processing
RHOA	131734	–1.42	5.36	Cell cycle/proliferation
ZNF131	24905	–1.45	5.36	Transcription regulation
–	24452	–1.45	5.36	Unknown
DCTN4	139390	–1.45	5.36	Unknown
SEPT6	143966	–1.47	5.36	Cell cycle/proliferation
FBXL14	21570	–1.52	5.36	Ubiquitin cycle
CABLES2	50389	–1.54	5.36	Regulation of cell cycle
ACSL4	133988	–1.56	5.36	Lipid biosynthesis and fatty acid degradation
INPPL1	142382	–1.58	5.36	Phosphate metabolism/intracellular signaling
TRHDE	37609	–1.59	5.36	Enzyme activity
KIAA0828	21593	–1.68	5.36	
RAD52	1675900	–1.68	5.36	DNA repair

we applied a variance filter in the first step to filter the genes with low variation (flat genes) between the control and exposed groups of individuals. In the second step, the SAM (Significance Analysis of Microarray)[26] method was applied to the data set. To verify whether the gene expression profiles can distinguish the occupationally exposed individuals from the unexposed individuals, a class prediction analysis was performed using the Supporting Vector Machine classification (SVM) and the K-nearest neighbor (KNN) classification methods included in MEV (v. 3.1) software.[25] The Principal Component Analysis (PCA) was also performed, and a 3-D view of the PCA plots for all the samples was generated. Genes that show significantly elevated expression included *SLC45A4*, *ADIPOR2*, *CCL4*, *HBB*, *HBA1*, *PACRG*, IMAGE38975, *LIG3* and *R3HDM1*. The genes that showed repressed expression included *ACAT2*, *VAMP1*, *ERCC5*, *GSTP1*, *HS6ST3*, *HNRPF*, *TMEM138*, *ACP6*, *PRAGMIN*, *KIAA0415*, *SLC25A25 and C2orf44*. In SVM, all the samples were used and the prediction accuracy was estimated by leave-one out Iterative validation. In KNN classification, 50% of the radiation exposed and control samples were used to train the algorithm, while the remaining 50% were left to be classified after training. In this case, the accuracy was also tested by the leave-one out cross-validation.

Results regarding gene location and biological functions were obtained at S.O.U.R.C.E. (http://genome-www5.stanford.edu/cgi-bin/SMD/source/source) and NCBI (http://www.ncbi.nlm.nih.gov/). Furthermore, modulated genes were divided by functional groups (Gene ontology, biological process) using DAVID.[27]

Quantitative real-time PCR (qPCR)

Quantitative real-time PCR was used to confirm gene expression profiles for six genes (*XPA*, *ERCC5*, *LIG3*, *SEPT6*, *DUSP22* and *RHOA*). The reverse transcription step was carried out with the Superscript III Reverse Transcriptase kit (Invitrogen) according to the manufacturer's instructions. The integrity of cDNA samples was validated by the amplification of the endogenous actin-β (ACTB) gene and visualization in agarose gel electrophoresis. qPCR was carried out using SYBR green master mix (Applied Biosystems) and the expression was estimated by ΔΔCt method.[28] Each reaction

Table 3. Prediction of exposed and un-exposed individuals using the expression values of the most significant 21 genes found by the SAM analysis. The classification was performed by the Supporting Vector Machine (SVM) and the K-nearest neighbor (KNN) analysis methods. Validation was carried out using the Leave One-Out Cross Validation (LOOCV) method.

SVM			
	Overall accuracy: 95.6%		
LOOCV	Initial Classification	Final Classification	
Class 1 (exposed)	E1–E14	E1–E14, C1	
Class 2 (control)	C1–C9	C2–C9	
KNN			
	Overall accuracy: 100% (train/classify approach)		
Training set	class 1 (exposed)	class 2 (control)	
	E1–E7	C1–C5	
Classified set			
	E8–E14	C6–C9	
	Overall accuracy: 83.3% (training set)		
LOOCV (training set)	Number of training set elements	Training set elements correctly assigned	Training set elements Falsely assigned
Class 1 (exposed)	7	7	2
Class 2 (control)	5	3	0
	Overall accuracy: 91.3% (all samples)		
LOOCV (all samples)	Number of training set elements	Training set elements correctly assigned	Training set elements Falsely assigned
Class 1 (exposed)	14	13	1
Class 2 (control)	9	8	1

had a total volume of 25 μL, containing 9 μL of water, 12.5 μL of SYBR Green, 1.25 μL (10 μM stock) of each forward and reverse primers (manufactured at Integrated DNA Technologies, USA) and 1 μL of cDNA obtained from RT-PCR reactions for each sample. The reactions were mounted in 96 wells polypropylene plates covered with microplate adhesives. The reactions were carried out in a Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems) using the following primer sets: *ACTB* for: 5'-GAT GAG ATT GGC ATG GCT TT-3' rev: 5'-ATT GTG AAC TTT GGG GGA TG-3'; *XPA* for: 5'-CAT CAT TCA CAA TGG GGT GA-3' rev: 5'-TTT TCT CGG TTT TCC TGT CG-3'; *ERCC5* for: 5'-GGG AAA CCT GAT CTC GAC AA-3' rev: 5'-AAT TCG GAG CTG TGT CTG CT-3'; *LIG3* for: 5'-GTA CGA TGG AGA GCG AGT CC-3' rev: 5'-AGC CTG GGG AAT GTA GTC CT-3'; *SEPT6* for: 5'GTC AGC AAC GGA GTC CAG AT-3' rev: 5'-TCT TCT GTG CTG CCA ATG AC-3'; *DUSP22* for: 5'-GGT CCA TCA GTA TCG GCA GT-3' rev: 5'-TCT TCT GAG AAA GGC CCA GA-3'; *RHOA* for: 5'-CGC TTT TGG GTA CAT GGA GT-3' rev: 5'-CAA GAC AAG GCA CCC AGA TT-3', with an annealing temperature near 60°C and an amplicon of 100–150 bp. The PCR cycle was the following: pre-heating at 50°C for 2 min., 10 min. at 95°C (denaturation step), followed by 40 cycles at 95°C for 15 sec., and at 60°C for 60 sec. The dissociation curves were set up as following: 95°C for 15 sec., 60°C for 20 sec. and 95°C for 15 sec. The comparison between control and exposed groups of samples were done by the t-test.

RESULTS

Microarray analysis revealed a total of 78 modulated genes in lymphocytes from occupationally exposed individuals (Table 2). The level of exposure (verified by physical dosimetry for the group of radiation workers) varied from 0.696 to 39.088 mSv, which is well within the admissible level for occupational exposure. Despite the low level of radiation, profound alterations in gene expression were observed in lymphocytes of the exposed individuals. The application of the t-test and the SAM statistical method showed a list of 78 differentially expressed genes (21 up-regulated and 57 down-regulated genes) and FDR < 5.4% was obtained for the exposed group compared to the unexposed control group.

The magnitude of changes observed for differentially expressed genes ranged from +2.5 to –1.8 (fold-change values). Among 78 genes, 21 up-regulated genes presented fold-changes from 1.2 to 2.5, while 57 down-regulated genes showed fold-change values between –1.14 to –1.80 (Table 2). However, the impact of the duration of employment time on gene expression could not be analyzed due to both restricted number of samples and variation in the employment period (1.5 to 17 years; mean ± SD = 9.32 ± 5.97).

Two classification analysis methods (SVM and KNN) were used to determine whether gene expression can distinguish the exposed from un-exposed individuals. A set of 21 induced and repressed genes (see Materials and Methods for more details) were chosen according to their significance to be used as classifiers. The selected genes can discriminate exposed from non-exposed individuals with an overall accuracy of 95.6% (SVM method), with only one control individual (C1) being misclassified. In KNN, 50% of samples were used to train the algorithm, while the remaining 50% were submitted to classification (Table 3). The KNN method successfully predicted the classes of all samples that were left out from the training procedure. The confidence of the classification was also measured by a leave one-out cross validation method (Table 3). Additionally, the samples were submitted to the PCA analysis, generating a 3-D view of PC plots for all samples (Fig. 1).

The real time PCR method was applied to validate the expression of six genes (*XPA*, *ERCC5*, *LIG3*, *SEPT6*, *DUSP22* and *RHOA*), using the same RNA samples that were used for gene expression experiments. The expression analysis showed an up-regulation of *XPA* and *LIG3*, and a down-regulation of *ERCC5*, *DUSP22, SEPT6* and *RHOA*, relatively to control levels (Fig. 2). Except for *LIG3*, the application of the t-test indicated significant differences between the control and exposed-group for *ERCC5* ($p = 0.02$), *DUSP22* ($p = 0.03$), *SEPT6* ($p = 0.04$), *RHOA* ($p = 0.02$), and *XPA* ($p = 0.06$) genes, thus confirming the results of the microarray analysis.

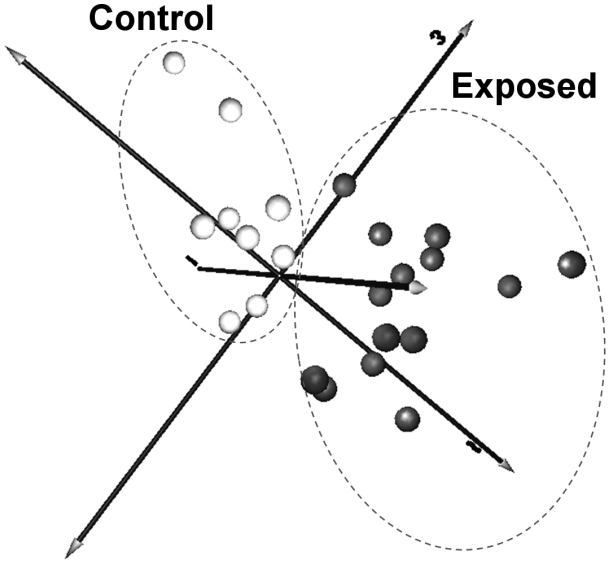

Fig. 1. Tridimensional score plot of first three principal components for exposed and un-exposed samples, according to the 21 most significant modulated genes. Dark markers represent the exposed individuals, while light markers represent the un-exposed individuals.

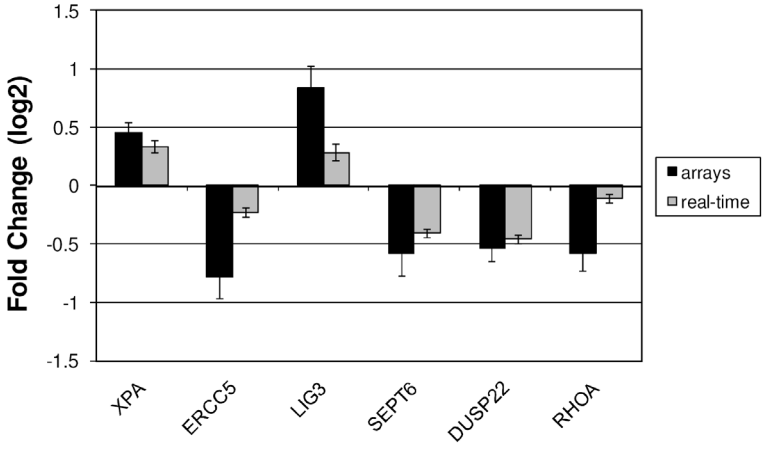

Fig. 2. Gene expression levels displayed by six genes (*XPA*, *ERCC5*, *LIG3*, *SEPT6*, *DUSP22* and *RHOA*) as determined by the real time PCR and the microarray methods, using the same RNA samples from radiation workers and control subjects. Except for *LIG3*, all four genes presented significant ($p < 0.05$) differences between control and exposed-group of samples, as evaluated by the t-test.

To find out the biological functions of the genes that showed differential expression, analysis was performed using DAVID. The most frequent GO categories of biological processes associated with the differentially expressed genes included cell cycle, intracellular signaling cascade, response to stress, apoptosis, ligase activity, ubiquitin cycle, DNA damage, DNA repair, helicase activity and proteolysis.

DISCUSSION

Evaluation of human health effects (immediate and delayed) after exposure to chronic low dose and dose rate has proven to be technically challenging for many decades. Cumulative radiation dose was found associated with 1.8-fold (SE = 0.9) increase in all-cancer mortality per 10 mSv, assuming a 10-year lag between radiation exposure and mortality.[29] Wilkinson and Dreyer[30] conducted an extensive epidemiological study, that showed an overall relative risk of 1.5-fold for leukemia (after adjustment of age and employment time) for radiation workers who presented cumulative occupational doses > 10 mSv (1 rem), compared to those who received < 10 mSv. An adjusted relative risk of 1.8-fold was observed for individuals exposed to 10–50 mSv (1–5 rem). Other effects (morphological alterations and functional dermal microcirculation) were found by capillary microscopy in occupationally exposed physicians (n = 145) to low-dose IR, lower than 5 rem/year (maximum permissible dose in Italy).[31]

Many important aspects should be taken into account in studies on gene expression, such as time of employment, total accumulated absorbed dose, age and life style, which were found relevant in cytogenetic studies.[32–34] In the present work, the radiation exposure along 1.5 to 17 years conferred an accumulated dose in a range of 0.696 to 39.088 mSv (which is within the admissible level for occupational exposure). For radiation workers, we found a list of 78 significantly altered genes (21 up-regulated and 57 down-regulated genes), for FDR < 5.4%. These alterations indicated that even low level exposure can induce stable transcriptional changes in the radiation workers.

A combination of genetic and non-genetic factors can be responsible for inter-individual variability observed among humans. In the present study, IR-exposed individuals were between 30 and 52 years-old when blood samples were collected, but the situation is that these individuals received a chronic low-dose exposure during different periods (mean: 9.32 ± 5.97 years) of employment. Another factor to be taken into account is the parallel induction of oxidative damage by many endogenous and exogenous agents, which may cause the development of age-related diseases, even during the normal life-span.[35] Since the control group was established according to age, gender and life style (alcohol drinking and smoking habit), such influences were minimized as much as possible in the analysis of gene expression.

The frequency of IR-induced chromosomal damages observed *in vivo* in human lymphocytes can be influenced by many factors: prolonged induction of DNA lesions, ongoing repair process and removal and re-distribution of circulating lymphocytes in the blood.[36] These aspects need to be considered for assessing the gene expression profiles observed in radiation workers. The integrity of eukaryotic genome is maintained by a coordinated network of cellular pathways. Several of these pathways have been found to be responsive to ionizing radiation exposure as determined by microarray analysis of gene expression. Genes commonly associated

with radiation response belong to multiple processes of DNA repair, cell cycle/proliferation, stress response and signaling transduction. Altered expression of a few genes playing specific roles in DNA repair/cell cycle control such as *CDKN1A*, *DDB2*, *XPC*, *GADD45A* and *PCNA* have been reported earlier.[26,37–39] In contrast, we did not find any alterations in any of the above mentioned genes in both radiation-exposed and unexposed human individuals. Variations observed between our study and others may be due to very low level of radiation exposure in the radiation workers. These variations can also be attributed to differences in cell types, experimental conditions, and different gene sets used in the microarray. Goldberg *et al.*[40] did not find a consistent dose-response or time-response pattern for some key genes (including *TP53*, *CDKN1A*, and *GADD45A*) analyzed in irradiated skin fibroblasts.

It is well-known that the maintenance of genomic integrity in mammalian cells depends on the presence of efficient DNA repair systems. Although mutations have long been identified as early events in carcinogenesis, defective DNA repair is also a risk factor for many types of cancer.[41] In this study we found transcriptional changes in as many as 5 genes (*LIG3*, *XPA*, *ERCC5*, *DCLRE1* and *RAD52*) that are involved in diverse DNA repair pathways. The expression levels of *ERCC5* ($p = 0.02$) and *XPA* ($p = 0.06$) genes were confirmed by quantitative real time PCR, although a borderline p value had been observed for *XPA*. Altered expression of DNA repair genes observed in radiation workers suggest the activation of DNA repair pathways in response to DNA damage/stress induced by chronic low radiation exposure. *LIG3* is involved in DNA repair and recombination, and two distinct isoforms, alpha and beta, encode polypeptides with different C-terminal amino acids. Employing a reconstituted BER complex, it was demonstrated that *LIG3* and *XRCC1* are essential mediators of BER pathway regulation.[42] Recently, Wang *et al.*[43] demonstrated that *LIG3* is a candidate for B-NHEJ, an alternative repair pathway of non-homologous end joining, indicating its multiple roles. The expression of *LIG3* gene was not confirmed by the PCR method ($p = 0.14$). *XPA* and *ERCC5* play a role in NER (nucleotide excision repair), which is the major DNA repair process that removes diverse DNA lesions including UV-induced photoproducts and chemical induced DNA cross-links. Recently, some of NER genes have been found differentially expressed in irradiated cells,[18,26,38] but the role of those genes in IR response is still unknown. Expression of NER genes in radiation workers seems to indicate their participation either in the repair of DNA strand breaks or in the repair of oxidative DNA damage. *ERCC5* (*XPG*) gene was found repressed in the radiation workers. XPG is a human endonuclease that incises 3' at DNA lesions during NER pathway. It is not clear at this point why *ERCC5* is down regulated in radiation workers.

DCLRE1C, DNA cross-link repair 1C (PSO2 homolog, S. cerevisiae), or Artemis, is a recently identified factor involved in V(D)J recombination and nonhomologous end joining (NHEJ) of DNA double-strand break (DSB) repair, but Artemis only partially participates in the NHEJ pathway to repair DSBs in human somatic cells.[44] Rad52 gene functions in homologous recombination repair. After radiation exposure, Rad52 forms *foci* in S and G2/M phases of the cell cycle.[45]

SEPT6 gene was found significantly down-regulated both by microarray and real time PCR analyses. This gene belongs to the GTP-binding protein family, and is involved in diverse processes including vesicle trafficking, apoptosis, cytoskeleton remodeling, infection, neurodegeneration and neoplasia.[46–48] Hall *et al.*[49] analyzed septin gene expression in different tissues, showing that many septins are expressed in all tissue types, but some of them show higher expression in lymphoid (SEPT1, 6, 9, and 12) or brain tissues (SEPT2-8 and 11). Two other down-regulated genes in radiation workers were *RHOA* and *DUSP22*, whose decreased expression levels were also confirmed by the real time PCR analysis. These two genes showed significant differences between control and exposed groups ($p < 0.03$). The protein encoded by *RHOA* is involved in cell proliferation/stress responses and belongs to Rho GTPases family that participate in cell growth, lipid metabolism, cytoarchitecture, membrane trafficking, transcriptional regulation and apoptosis in response to genotoxic agents. They trigger specific signals that lead to uncontrolled cell growth, enhanced angiogenesis, inhibition of apoptosis, and genetic instability, resulting in tumor development.[50] *DUSP22* gene acts preferentially on the c-JUN n-terminal kinase (JNK) and p38 MAPKs, playing a role in apoptosis and cell proliferation. Recent findings suggested that DUSP22 acts as a negative regulator of the ER alpha-mediated signaling pathway.[51] Some members of the DUSP family were previously reported as radiation-responsive in fibroblasts[52] and *in vitro* irradiated lymphocytes.[53] In fibroblasts, changes of gene expression were greater at 2 cGy (low dose) than at 4 Gy (high dose), suggesting that some cellular pathways may be more sensitive to low-dose irradiation.[40]

Changes in the transcriptional expression of many genes observed in radiation workers may lead to deregulation of several metabolic processes, which in turn increase the risk of carcinogenesis. The evaluation of low dose effects still represents a challenge in radiobiology. As a whole, the approach used in the present study is relevant in human population monitoring, in case of occupational or accidental exposure to IR. The expression profiles of 21 genes were found useful to distinguish the exposed from un-exposed individuals. This study raises the possibility of using these genes as biomarkers for assessment of low radiation exposure in humans. Additionally, these alterations detected in radiation workers illustrate a critical need for determining the safest radiation dose at which no observable biological

effects (immediate or late) are seen in human populations.

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