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Research Article

Effect of ionizing radiation on gene expression in CD4+ T lymphocytes and in Jurkat cells: unraveling novel pathways in radiation response

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Abstract. To better understand at the molecular level the effect of ionizing radiation in leukocytes, the global transcriptional response to X-ray irradiation was studied in human CD4+ T lymphocytes and in Jurkat cells. Microarray analysis performed on freshly isolated human CD4+ T lymphocytes 8 h after an LD50 irradiation dose of 1 Gy revealed that out of 13,825 genes, 1084 were modulated more than 1.5-fold. The most strongly up-regulated genes were predominantly p53 targets. In contrast, exposure of the CD4+ T lymphocyte-derived Jurkat leukemic cell line (with no functional *p53* gene) to an equivalent LD50 dose (0.5 Gy) induced a partly different and more limited set of genes. Interestingly, this set of genes belonged to the Rho and cytokine signaling pathways, suggesting the existence of novel pathways regulated by low-dose ionizing radiation.

Key words. X-ray; apoptosis; lymphocytes; gene expression; p53; Rho signaling.

Ionizing radiation activates a complex network of signaling pathways that mediate various cellular responses, such as transcriptional modulation of genes, cell cycle arrest and apoptosis [1]. These responses are likely to differ depending on several factors such as the type, differentiation or transformed state of the cell, mutations and polymorphisms in specific genes, phase of the cell cycle and the microenvironment (presence of growth and differentiation factors).

Radiation-induced alteration in gene expression has been documented in various cell types and experimental settings. In most cases, these studies have been limited to a few genes for which a priori hypotheses had been elaborated. Many of the induced genes encode transcription factors such as c-jun, p53, NF-κB and AP-1. Other genes modulated by radiation include *cyclin B1*, proliferating nuclear antigen, cytoskeleton elements, protein kinase C, and tumor necrosis factor alpha [2, 3].

However, these studies do not allow the drawing of a global picture of the cellular transcription response triggered by ionizing radiation. The development of genomic approaches has brought new tools to reveal the complexity of the various cellular responses to environmental agents. One of the first such genomic studies used to unravel the transcriptional response to ionizing radiation was performed on normal peripheral blood lymphocytes (PBLs) [4, 5]. In these cells, the major regulation was found to be linked to the activation of the *p53* gene, with mRNA levels of p53-regulated stress-response genes such as *CDKN1A*, *GADD45*, *cyclin G1* and *FHL2* increasing several-fold after radiation exposure [4, 5]. In contrast to normal cells in which ionizing radiation induces a strong transcriptional response, transformed cells

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seem to show a more limited response both in terms of number of regulated genes and amplitude of gene regulation [3, 6].

Even though only modest changes are seen at the transcriptional level in transformed cells following radiation exposure, cellular effects are present but they differ from those observed in normal cells. The complexity of the response varies according to the cell type. Lymphoid and myeloid cell lines undergo radiation-induced apoptosis with different time courses depending on the cell line. Apoptosis occurs either rapidly, within a few hours after exposure (interphase death) or after a G2 phase prolonged block (delayed interphase death) or even after the first and the following mitoses (mitotic death) [7].

In the present study, we used high-density microarrays to compare the transcriptional response to radiation of freshly isolated CD4+ T cells and of the Jurkat leukemic cell line (derived from CD4+ T cells). Different signaling pathways specific for the two cell types were tentatively identified, including a novel signaling pathway mediated by the Rho GTPase which is down-regulated after irradiation in Jurkat cells.

Materials and methods

Cell purification, culture and irradiation

Peripheral blood mononuclear cells (PBMCs) were isolated from the venous blood of healthy donors by density centrifugation over Ficoll Paque (Sigma, St. Louis, Mo., density 1077 g/ml). CD4+ T lymphocytes were purified from PBMCs using super-paramagnetic anti-CD4 MACS MicroBeads antibody (Miltenyi Biotec, Bergisch Gladbach, Germany), as suggested by the manufacturer.

Freshly isolated lymphocytes or Jurkat cells were cultured in RPMI 1640 medium (Invitrogen, Merelbeke, Belgium) supplemented with 10% fetal bovine serum (FBS). Irradiation was performed 24 h after blood collection or cell plating with X-rays (250 kV, 15 mA, 1 mm Cu) at a dose rate of 0.3 Gy/min.

Flow cytometry analysis

Flow cytometry analyses were performed on an Epics XL fluorescence-activated cell sorter (Coulter Beckman, Fullerton, Calif.).

Population purity was controlled by labeling primary lymphocytes with the fluorescein-coupled anti-human CD4-specific antibody [mouse IgG1; Analis, Namur, Belgium; diluted ten times in phosphate-buffered saline (PBS) with 0.5% FBS] for 20 min.

DNA content and cell cycle distribution were measured as previously described [8]. Reactive oxygen species (ROS) measurement was made after incubating cells in PBS containing 0.125 µM dihydroethidium (Molecular Probes, Eugene, Oreg.) for 10 min at 37 °C.

RNA isolation and quantitative real-time RT-PCR analysis

RNA was extracted in Trizol and analyzed by quantitative real-time reverse transcription PCR as previously reported [8].

Microarray analysis

Five micrograms of total RNA was reverse transcribed with an oligo-dT primer linked to a T7 promoter sequence and linearly amplified using an in vitro transcription reaction as previously described [8, 9]. The amplified RNA was used to label the cDNA with Cy3 (sham control) or Cy5 (irradiated), which was subsequently hybridized to three separate glass slides each containing an average of 4,300 unique cDNAs spotted in duplicate. Microarray experiments and subsequent analysis followed the procedures previously described [8].

Results

Genes modulated by ionizing radiation in primary CD4+ T lymphocytes

The cellular response of normal CD4+ T lymphocytes to ionizing radiation was first characterized as a function of dose and time in cells freshly isolated from three healthy donors (fig. 1a, b). The fraction of apoptotic (sub-G1) and ROS producing cells increased gradually after 24 h to be maximal at 48 h. This increase was statistically significant only for the dose of 1 Gy. The expression of the DNA damage induced-binding protein DDB2, known to be positively regulated in PBLs after irradiation [4], was strongly activated with a peak at 8 h (11.4-fold after 1 Gy radiation compared to the sham-irradiated cells), preceding cellular effects (fig. 1c). The activation of *DDB2* studied at 8 h after 1 Gy radiation in the other two donors was also increased by 4.7- and 9-fold, compared to the sham-irradiated cells.

This time point and a dose of 1 Gy corresponding to the LD50 [10] were chosen to investigate the global profile of gene expression with microarrays containing 13,825 cDNAs. The total number of genes modulated more than

Table 1. Summary of genes modulated by ionizing radiation in primary CD4+ T lymphocytes.

Tested genes	13,825		
Total modulated genes	1084 (7.8%)		
Activated genes	736 (5.3%)	Repressed genes	348 (2.5%)
Fold change		Fold change	
Over 3.5	11	Over 3.5	0
Between 2 and 3.5	75	Between 2 and 3.5	8
Between 1.5 and 2	650	Between 1.5 and 2	340

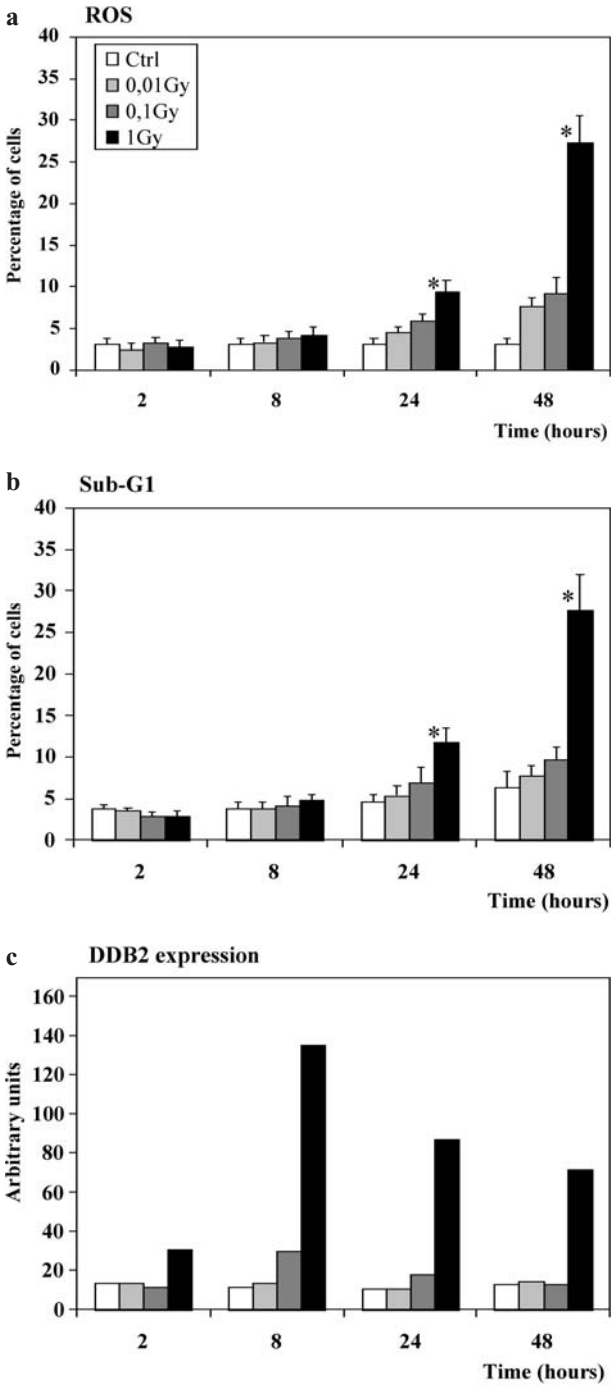

Figure 1. Effect of various doses of radiation in CD4+ T lymphocytes compared as a function of time. Values represent the mean ± SE of three independent donors. (*a*, *b*) Flow cytometry analysis showing the percentage of cells positive for ROS and the sub-G1 population. (*c*) RT-PCR on the *DDB2* gene (one of the three donors). * Indicates p values smaller than 0.05.

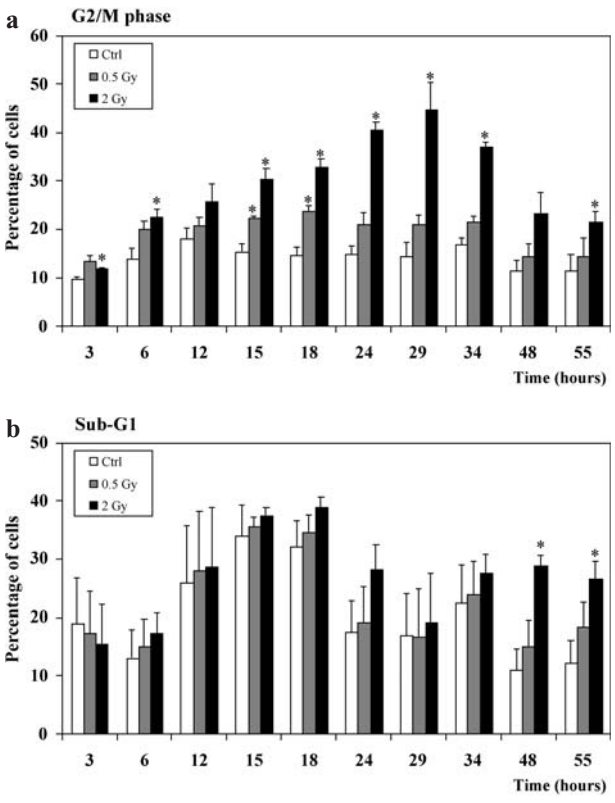

Figure 2. Cell cycle distribution of Jurkat cells irradiated with 0.5 and 2 Gy over 55 h. The values are given as percentage of cells in the sub-G1 or G2/M phase. Values represent the mean ± SE of four independent experiments. * Indicates p values smaller than 0.05.

1.5-fold by radiation is summarized in table 1. The level of modulation was up to 7.4-fold for activation and up to 3.4-fold for repression. Among the 11 most activated genes (over 3.5-fold up-regulation), 7 (*GADD45A*, *DDB2*, *PCNA*, *DRAL/FHL2*, *TNFRSF10B*, *CDKN1A*, *SESN1*) were p53 targets (table 2). Three other known p53 targets (*BAX*, *ATF3* and *CD95/FAS*) were also activated albeit at a lower level. The expression of seven out of the ten p53 targets was validated by quantitative RT-PCR (table 2). Globally, the values from the microarrays were slightly underestimated if compared to those from RT-PCR. Few genes were repressed, among which the most down-regulated one was *MYC* (2-fold).

Only few genes are regulated in both primary CD4+ T lymphocytes and Jurkat cells

To determine whether a similar pattern of gene regulation was observed in cancer cells, the cellular response to irradiation was compared in the Jurkat leukemic cells. Jurkat cells were irradiated with 0.5 Gy, corresponding to the LD50 [11] or 2 Gy (corresponding to the daily dose conventionally delivered by radiotherapy). As shown in figure 2, cells arrested in the G2/M phase with a peak between 24 and 34 h. The proportion of dead cells (in the sub-G1 region) increased slightly only after 34 h, with a maximum increase at 48 and 55 h. The effect was significant only after irradiation at 2 Gy.

On the basis of these biological effects, microarrays were performed in cells irradiated with 0.5 Gy and 2 Gy at 12

Table 2. p53 TARGETS modulated more than 1.5-fold by radiation in freshly isolated CD4+ lymphocytes.

Short name	Gene Bank Identifier	Array	qPCR	Description and function
GADD45A	N98621 AI935984	7.4 1.7	13.9	growth arrest and DNA damage-inducible, alpha; the protein responds to environmental stresses by mediating activation of the p38/JNK pathway.
DDB2	NM_000107 R01076	5.4 5.7	8.5	damage-specific DNA-binding protein 2; activated by UV and X-ray irradiation; it recognizes pyrimidine dimers and increased p53 expression.
PCNA	T82974	4.6	nd	proliferating cell nuclear antigen; it associates with histone deacetylase; involved in chromatin remodeling.
DRAL/*FHL2*	W46835	4.2	nd	four-and-a-half LIM domains 2; binds to integrins; recruited to adhesion complexes; transcriptional corepressor of histone deacetylase; involved in chromatin remodeling.
TNFRSF10 B (*DR5*)	H68181	4.1	11.7	tumor necrosis factor (TNF) receptor superfamily, member 10b; transduces apoptosis signal after binding with TRAIL.
SESN1	N68917 AI572037	3.6 2.9	12.4	p53-regulated PA26 nuclear protein; involved in cell cycle arrest.
CDKN1A	R87514 L26165 W39472	3.5 2.1 1.7	8.9	cyclin-dependent kinase inhibitor 1A (p21, Cip1); induced by BRCA1 protein.
BAX	AI025937	2.3	10.7	BCL2-associated X protein; forms a heterodimer with BCL2, and functions as an apoptotic activator.
ATF3	N69679	1.9	10.1	activating transcription factor 3, member of the CREB family; ATF3 represses transcription from promoters with ATF-binding elements.
TNFRSF6 (*Fas*)	AA406323	1.7	nd	TNF receptor superfamily, member 6; the protein plays a central role in the physiological regulation of programmed cell death.

Levels of transcriptional modulation are indicated for both microarray and quantitative RT-PCR experiments (qPCR). Some genes were represented more than once in the array and values are given for each probe. nd, not done.

and 23 h. In contrast to CD4+ T lymphocytes, few genes (322) were modulated in any of the four conditions. The magnitude of the modulation reached 6.5-fold for up-regulation and 6.6-fold for down-regulation, although the modulation of around 96% of the genes was between 1.5- and 2.5-fold. Generally, the magnitude of the activation was lower in Jurkat cells than in freshly isolated CD4+ T lymphocytes and the reverse was observed for repression. As shown in figure 3, the expression of 46 genes only (0.33% of the total) was modulated in both Jurkat and primary CD4+ T lymphocytes. Those genes are listed in table 3. Among them, 34 were modulated in the same direction. Three activated genes were known p53 targets (*ATF3*, *DDB2* and *GADD45A*) but their levels of activation were weak.

Transcriptional response associated with radiation exposure in Jurkat cells

To understand the molecular alterations following irradiation in Jurkat cells, the analysis of the 322 modulated genes was further refined. In a first step, only the genes whose transcription was modulated by irradiation in the same way irrespective of the dose were considered, yielding a cluster of 59 genes, of which 42 were up-regulated and 17 down-regulated (table 4). This set included genes encoding transcription factors (*ATF1*, *ATF6*, *NFKBIB*, and *TRIM8*), cell cycle regulators (*ANAPC1*, *GADD45A*, *CDC5L*, *STK38* and *spermidine synthetase*), proteins involved in Ras (*RRAGC*) and Rho signaling (*ARHG*), ribosomal and translational elongation genes (*RPS8, EEF1A1*) and stress proteins (*PPP6C*). These genes might represent general indicators of radiation exposure.

Novel dose-dependent pathways possibly leading to changes in the cytoskeleton and proliferation

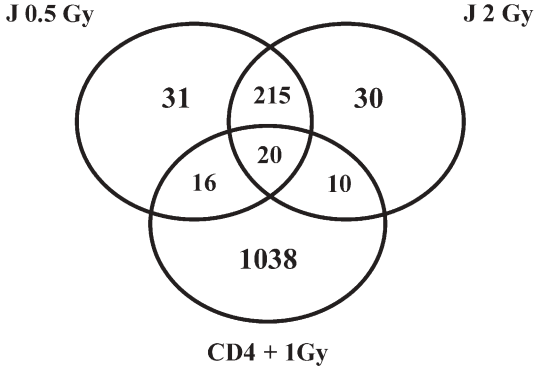

Figure 3. Venn diagram indicating the number of genes commonly modulated more than 1.5-fold by irradiation in primary CD4+ T lymphocytes and the Jurkat T cell line out of a total of 13,825 cDNAs on the microarrays.

Table 3. Genes modulated by irradiation in both primary CD4+ T lymphocytes and Jurkat cells

Short name	Gene Bank Identifier	Description	Primary T cells	Jurkat cells			
			1 Gy for 8 h	0.5 Gy for 12 h	0.5 Gy for 23 h	2 Gy for 12 h	2 Gy for 23 h
Modulation in the same way							
UP-REGULATED							
CD4+ T lymphocytes and Jurkat cells (0.5 and 2 Gy)							
CTL2	N72581	*CTL2* gene	2.7	1.5	2.5	1.3	1.5
GADD45A	N98621	growth arrest and DNA damage-inducible, alpha	7.4	1.5	1.6	1.5	2.1
RPS6KA5	H09985	ribosomal S6 kinase, polypeptide 5	1.7	2.1	2.7	3.4	2.6
STK18	H21657	serine/threonine kinase 18	1.6	2.4	2.4	3.9	1.3
TRIM8	H15926	tripartite motif-containing 8	1.7	1.8	1.9	2.5	1.8
	R48133	hypothetical protein FLJ11267	1.9	1.7	1.8	1.8	1.4
CD4+ T lymphocytes and Jurkat cells (0.5 Gy)							
C6orf35	AA167536	chromosome 6 ORF 35	1.6	1.5	1.3	1.1	1.2
LYAR	BG190818	hypothetical protein FLJ20425	2.6	1.6	1.2	–1.1	–1.1
PLEK	NM_002664	pleckstrin	1.7	–1.2	1.7	1.1	1.1
RPL36A	R05264	ribosomal protein L36a	1.5	1.2	1.6	1.1	1.1
CD4+ T lymphocytes and Jurkat cells (2 Gy)							
ATF3	N69679	activating transcription factor 3	1.9	1.4	1.4	1.3	2.4
DDB2	R01076	damage-specific DNA-binding protein 2	5.7	1.2	1.4	1.1	1.8
MAP4K4	BE350101	mitogen-activated protein kinase kinase kinase kinase 4	1.7	1.4	1.3	1.5	1.1
RPS27L	AA046808	ribosomal protein S27-like	4.7	1.2	1.3	1.1	1.6
SRPK2	R78795	SFRS protein kinase 2	1.9	1.3	1.2	1.6	1.0
DOWN-REGULATED							
CD4+ T lymphocytes and Jurkat cells (0.5 and 2 Gy)							
AAAS	T83213	ALADIN (achalasia, adrenocortical insufficiency, alacrimia)	–1.5	–2.5	–2.2	1.1	–1.5
ARHGDIA	R20223	Rho GDP dissociation inhibitor (GDI) alpha	–1.5	–2.3	–2.3	–1.6	–2.4
B3GAT3	R56054	beta-1,3-glucuronyltransferase 3	–1.5	–2.3	–2.1	–1.7	–1.5
CSK	H61706	src tyrosine kinase	–1.5	–2.1	–2.0	–1.1	–1.9
RAVER1	R18466	RAVER1	–1.6	–3.0	–3.0	–1.8	–2.0
SMYD5	BG333600	SMYD family member 5	–1.7	–2.4	–2.1	–1.2	–1.6
STAT5B	T83012	signal transducer and activator of transcription 5B	–1.5	–1.7	–1.4	–1.5	–1.2
TCFL1	AI366716	transcription factor-like 1	–1.1	–2.0	–1.4	–1.9	–1.0
	R88064		–1.6	–1.4	–1.3	–1.1	–1.1
TOMM40	N20321	translocase of outer mitochondrial membrane 40 homolog	–1.8	–1.9	–1.9	–1.1	–1.5
TRIM28	R94221	tripartite motif-containing 28	–1.5	–2.6	–2.0	–1.1	–1.8
	H29049	EST	–1.7	–2.5	–2.2	–1.3	–1.5
CD4+ T lymphocytes and Jurkat cells (0.5 Gy)							
ARHGEF1	R56393	Rho guanine nucleotide exchange factor (GEF) 1	–1.8	–2.7	–2.4	–1.2	–1.3
CNN2	T97947	calponin H2	–1.8	–1.9	–1.6	1.1	–1.3
FLJ13868	NM_022744	hypothetical protein FLJ13868	–1.6	–2.2	–2.0	–1.3	–1.3
GDI1	H51215	GDP dissociation inhibitor 1	–1.6	–2.6	–2.1	1.2	–1.4
MYC	BG256267	v-myc myelocytomatosis viral oncogene homolog	–2.1	–1.8	–1.5	–1.2	–1.1
RGS14	N71460	regulator of G protein signaling 14	–1.8	–2.1	–2.0	–1.2	–1.2
	AF040753	EST	–1.5	–2.2	–2.0	–1.0	–1.3
CD4+ T lymphocytes and Jurkat cells (2 Gy)							
NRAS	BC005219	neuroblastoma RAS viral oncogene homolog	–1.6	1.1	1.1	1.2	–2.1
Modulation in opposite ways							
Up-regulated in CD4+ T lymphocytes and down-regulated in Jurkat							
Down-regulated at 0.5 and 2 Gy							
DNAJB5	N80249	DnaJ (Hsp40) homolog	1.6	–2.3	-2.6	1.2	–1.7
	T79637	EST	2.4	–1.7	–1.4	–2.0	–2.0
Down-regulated at 0.5 Gy							
ALG12	AI347229	alpha-1,6 mannosyltransferase	2.6	–1.9	–1.9	1.1	1.2
MLF2	BE869155	myeloid leukemia factor 2	1.7	–2.1	–1.7	1.1	–1.1
RAP1GA1	R60136	RAP1, GTPase-activating protein 1	1.7	–1.8	–1.2	1.3	–1.0
SGT	BE252550	small glutamine-rich tetratrico peptide repeat (TPR)-containing, alpha	1.6	–1.9	–1.7	–1.1	1.0

Table 3 (continued)

Short name	Gene Bank Identifier	Description	Primary T cells	Jurkat cells			
			1 Gy for 8 h	0.5 Gy for 12 h	0.5 Gy for 23 h	2 Gy for 12 h	2 Gy for 23 h
Down-regulated at 2 Gy							
NDUFB10	BE391452	NADH dehydrogenase1 beta subcomplex, 10	1.8	–1.2	1.0	–1.9	1.2
Down-regulated in CD4+ T lymphocytes and up-regulated in Jurkat Up-regulated at 0.5 and 2 Gy							
SEMA4C	R54587	semaphorin 4C	–1.5	1.8	1.9	1.4	1.0
Up-regulated at 0.5 Gy							
C5orf5	N59606	chromosome 5 ORF 5	–1.5	1.9	1.4	–1.2	1.4
Up-regulated at 2 Gy							
ILF3	AF007140	interleukin enhancer-binding factor 3	–1.7	1.1	1.0	1.5	–1.1
SLC1A5	BF206100	neutral amino acid transporter, member 5	–1.6	–1.4	–1.4	1.5	–1.0
	NM_003177	EST	–1.6	1.0	–1.2	1.6	–1.3

Table 4. Genes modulated in the same direction by ionizing radiation (0.5 and 2 Gy) in Jurkat cells.

Short name	Gene Bank identifier	Description	0.5 Gy 12 h	0.5 Gy 23 h	2 Gy 12 h	2 Gy 23 h
Up-regulated						
ANAPC1	H08575	anaphase-promoting complex 1	2.0	1.7	1.7	1.5
ATF1	R71081	activating transcription factor 1	2.3	2.2	3.7	1.7
ATF6	H26596	activating transcription factor 6	1.9	1.9	2.6	1.5
B2M	T85545	beta-2-microglobulin	1.7	1.8	2.0	1.5
BNIP3L	AI126040	BCL2/adenovirus E1B 19 kDa interacting protein 3-like	1.5	1.2	1.3	1.2
	R26460		1.5	1.4	1.5	1.5
C10orf2	R19418	chromosome 10 ORF 2	1.8	2.3	2.1	1.7
CNK1	T86626	connector enhancer of KSR-like (suppressor of Ras)	1.6	2.2	2.3	1.6
DKFZP434I116	H27254	DKFZP434I116 protein	1.6	2.0	2.2	1.7
EEF1A1	T71251	eukaryotic translation elongation factor 1, alpha	2.3	2.4	3.0	1.6
FEM1B	R12189	fem-1 homolog b	2.1	2.1	2.5	1.9
FLJ10110	H08091	hypothetical protein FLJ10110	1.6	1.7	2.1	1.6
FLJ23323	T79835	hypothetical protein FLJ23323	2.1	2.1	2.9	1.7
*GADD45A**	N98621	growth arrest and DNA damage-inducible, alpha	1.5	1.6	1.5	2.1
GOSR2	R18689	Golgi SNAP receptor complex member 2	1.7	1.7	1.9	1.8
HPS4	R27707	Hermansky-Pudlak syndrome 4	1.6	1.8	1.8	1.6
IFRD1	T97762	interferon-related developmental regulator 1	1.9	1.9	1.8	1.6
KIAA0446	T97299	KIAA0446 gene product	1.9	2.2	1.9	1.7
KIAA1033	R53810	KIAA1033 gene product	2.8	3.0	6.5	2.2
KIAA1387	R62828	KIAA1387 gene product	2.3	2.2	3.2	1.8
KLF7	H25429	Kruppel-like factor 7	2.5	2.2	4.3	2.0
LARS	R32993	leucyl-tRNA synthetase	2.2	2.4	2.0	1.7
LOC51257	AF070529	hypothetical protein LOC51257	1.8	2.5	2.8	1.7
NET1	R25091	neuroepithelial cell-transforming gene 1	1.7	1.7	2.2	1.7
NR5A2	R10138	nuclear receptor subfamily 5, group A	2.3	2.4	2.9	2.0
PNN	R15752	pinin, desmosome-associated protein	2.3	2.1	2.6	2.0
PPAP2A	R39256	phosphatidic acid phosphatase type 2A	2.0	2.1	2.3	1.6
PPP6C	R21669	protein phosphatase 6, catalytic subunit	1.7	1.9	2.0	1.5
RNASE6PL	R67645	ribonuclease 6 precursor	1.6	2.0	1.7	1.8
*RPS6KA5**	H09985	ribosomal protein S6 kinase, polypeptide 5	2.1	2.7	3.4	2.6
RPS8	T79482	ribosomal protein S8	1.7	2.6	2.3	1.9
RRAGC	T81311	Ras-related GTP-binding C	1.8	2.3	2.1	1.7
STK38	R20152	serine/threonine kinase 38	1.7	2.0	2.5	1.5
*TRIM8**	H15926	tripartite motif-containing 8	1.8	1.9	2.5	1.8
TUBGCP3	R07480	tubulin, gamma complex associated protein 3	2.6	2.7	3.2	1.5
TWSG1	H13430	twisted gastrulation homolog 1	1.9	2.3	3.6	1.9
UBE1	R72762	ubiquitin-activating enzyme E1	1.9	2.1	2.3	1.7

Table 4 (continued)

Short name	Gene Bank identifier	Description	0.5 Gy 12 h	0.5 Gy 23 h	2 Gy 12 h	2 Gy 3 h
UBN1	R12851	ubinuclein 1	1.9	2.4	3.3	1.9
ZBTB1	R26461	zinc finger and BTB domain-containing 1	2.3	2.8	3.5	2.2
	R60556	Hs transcribed sequence	1.9	1.9	2.1	1.5
	H15751	Hs transcribed sequence	1.5	1.5	2.2	1.5
	T96961	KIAA0492 gene product	2.7	2.2	3.8	1.7
	H05110	Hs transcribed sequence	2.5	2.6	2.9	1.8
Down-regulated						
ARHG	BG338917	Ras homolog gene family, member G (rho G)	–2.5	–1.7	–2.6	–1.2
	R52487		–2.2	–1.8	–1.9	–1.7
ARRB2	T97711	arrestin, beta 2	–2.6	–1.9	–1.8	–1.8
B3GAT3	R56054	beta-1,3-glucuronyltransferase 3	–2.3	–2.1	–1.7	–1.5
CDC5L	R34903	CDC5 cell division cycle 5-like	–1.7	–1.7	–1.8	–2.0
DKFZP566A1524	R56089	hypothetical protein DKFZp566A1524	–4.3	–3.6	–5.8	–2.6
MRF-1	R25557	modulator recognition factor I	–2.0	–1.8	–1.7	–2.0
NAPA	R07025	N-ethylmaleimide-sensitive factor attachment protein, alpha	–2.4	–4.0	–1.7	–1.6
	BF663204		–2.0	–1.6	–1.1	–1.9
NFKBIB	R09184	nuclear factor of kappa light polypeptide gene enhancer in	–1.7	–5.1	nd	–2.1
	AI935157	B cells inhibitor, beta	–1.6	–1.9	–1.3	–1.1
NFRKB	R23735	nuclear factor related to kappa B-binding protein	–2.1	–1.7	–1.8	–1.4
NINJ1	T84142	ninjurin 1	–2.2	–1.8	–2.7	–2.7
OGDH	R32729	oxoglutarate dehydrogenase	–2.7	–2.1	–2.4	–2.0
PRKCSH	R17511	protein kinase C substrate 80 K-H	–3.5	–2.4	–1.8	–1.7
RAVER1	R18466	RAVER1	–3.0	–3.0	–1.8	–2.0
SCARB1	T97259	scavenger receptor class B, member 1	–2.0	–1.7	–2.0	–2.7
SLC12A7	T81795	solute carrier family 12, member 7	–2.1	–1.7	–6.6	–1.5
SRM	R48806	spermidine synthase	–1.9	–1.9	–1.7	–1.6
TRAP95	H16593	thyroid hormone receptor-associated protein	–2.1	–2.1	–2.5	–1.9

nd, not done; * Also modulated in CD4+ T cells.

In a second step, the genes expressed in a dose-specific response were taken into consideration. A total of 47 and 40 genes were specifically modulated by 0.5 and 2 Gy, respectively (fig. 3). Most of the genes modulated with 0.5 Gy are down-regulated. Interestingly, this cluster included genes involved in Rho GTPase signaling (*RGS14, ARHGEF1*) and genes under the control of this pathway, such as cytoskeletal proteins (actin, spectrin, SWI/SNF proteins), and those associated with cytokine signaling (*IL2RG*, *IRF1, STAT6*) (table 5). An additional 13 genes known to participate in Rho GTPase signaling but not specifically repressed by 0.5 Gy, are also included in table 5. Not surprisingly, most of these genes were repressed by 2 Gy in addition to being repressed by 0.5 Gy. They included the Rho GTPase genes (*ARHG, RGS19IP1, ARHGDIA*), cytoskeletal genes (arrestin, katanin, additional SWI/SNF proteins, adhesion molecules), the inhibitor of the transcription factor NFκB (*NFkBIB*) and genes associated with STAT signaling (*STAT5B*). Some of these effects were also found in CD4+ T lymphocytes (table 3 and data not shown). The expression of 11 down-regulated genes was validated by quantitative RT-PCR (table 5) with the same RNA used for the microarray. Their level of transcription (excluding *STAT5B* and *STAT6*) was verified by quantitative RT-PCR in at least one independent experiment (data not shown). With the exception of *IL2RG*, all genes were confirmed to be down-regulated.

Discussion

In the present study, using microarray technology, we compared for the first time the expression of a large set of genes in freshly isolated CD4+ T lymphocytes (which express a functional p53 protein) and a T leukemic cell line (with no functional p53) following radiation exposure. Because the two cell types respond differently to irradiation, the irradiation doses under investigation with the microarray experiments were the LD50.

Ionizing radiation (1 Gy) induced apoptosis in primary CD4+ T lymphocytes, as indicated by the cytoplasmic accumulation of ROS and DNA fragmentation. Subsequent analysis by microarray confirmed the activation of the p53-dependent cell death program at the genomic level with the up-regulation of pro-apoptotic p53 target genes, such as the TRAIL receptor *TNFRSF10B*, *BAX* and *CD95/FAS.* The most strongly modulated genes were *GADD45A* and *DDB2*, both p53 targets involved in DNA repair [12, 13]. These findings have also been made in

Table 5. Genes from Rho and cytokine signaling pathways modulated by 0.5 Gy radiation in Jurkat cells.

Short name	Gene Bank identifier	Description	0.5 Gy 12 h	0.5 Gy 23 h	2 Gy 12 h	2 Gy 23 h
Specific for 0.5 Gy						
Up-regulated						
ARHGEF9	T83232	Cdc42 guanine nucleotide exchange factor 9	*1.7*	*1.6*	1.0	1.2
IL16	R17227	interleukin 16	*1.6*	–1.1	–1.2	1.0
Down-regulated						
ACTA1	N70860	actin, alpha 1, skeletal muscle	*–2.1*	*–1.9*	–1.0	–1.4
ACTA2	NM_001613	actin, alpha 2, smooth muscle, aorta	*–2.3*	*–1.6*	–1.2	–1.0
ACTB	R23540	actin, beta	*–2.2* (–3.6)*	*–2.1*	–1.2	–2.2
		actin, beta	*–2.4*	*–2.2*	–1.1	–1.1
		actin, beta	*–2.1*	*–2.1*	–1.0	–1.0
ARHGEF1	BE397399	Rho guanine nucleotide exchange factor 1	*–2.4*	*–2.3*	1.2	nd
	R56393		*–2.7* (–1.8)*	*–2.4*	–1.2	–1.3
GDI1	H51215	GDP dissociation inhibitor 1	*–2.6* (–1.7)*	*–2*.1	1.2	–1.4
IL2RG	NM_000206	interleukin 2 receptor, gamma chain	*–2.1*	*–1.5*	1.1	–1.1
	W61265		*–2.6* (–1.7)*	*–1.6*	–1.3	–1.2
IRF1	NM_002198	interferon regulatory factor 1	*–1.8*	–1.4	–1.3	1.1
	W39703		*–1.7* (–1.5)*	–1.4	–1.0	–1.2
RGS14	N71460	regulator of G protein signaling 14	*–2.1*	*–2.0*	–1.2	–1.2
SMARCF1	BF808164	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily f, member 1	*–1.2*	*–2.1*	–1.3	nd
SPTAN1	AL110273	spectrin, alpha, non-erythrocytic 1	*–2.3*	*–1.7*	1.2	–1.0
	R14737		*–2.9*	*–2.2*	–1.5	–1.5
STAT6	H14811	signal transducer and activator of transcription 6	*–1.8* (–1.8)*	*–1.8*	–1.2	–1.2
Genes relevant for the novel pathway but not specific for 0.5 Gy						
ADRM1	BF033463	adhesion-regulating molecule 1	–2.5	–2.0	–1.5	–1.3
	R13328		–2.5	–1.8	–1.5	–1.8
AKT3	R54092	v-akt murine thymoma viral oncogene 3	1.6	1.6	1.5	1.3
CNN2	AU124309	calponin H2	–2.3	–1.8	–1.8	–1.5
	T97947		–1.9 (–1.7)*	–1.6	1.1	–1.3
PDPK1	H11097	3-phosphoinositide-dependent protein kinase-1	1.8	1.8	2.1	1.4
ARHG	BG338917	Ras homolog gene family, member G (rho G)	–2.5	–1.7	–2.6	–1.2
	R52487		–2.2 (–1.7)*	–1.8	–1.9	–1.7
ARHGDIA	R20223	Rho GDP dissociation inhibitor alpha	–2.3 (–2.0)*	–2.3	–1.6	–2.4
ARRB2	T97711	arrestin, beta 2	–2.6 (–1.5)*	–1.9	–1.8	–1.8
KATNB1	NM_005886	katanin p80 subunit B 1	–2.2	–1.8	–2.1	–1.0
	R00741		–2.4	nd	–3.9	–1.2
NFKBIB	R09184	nuclear factor of kappa light polypeptide gene enhancer	–1.7	–5.1	nd	–2.1
	AI935157	in B-cells inhibitor, beta	–1.6	–1.9	–1.3	–1.1
RGS19IP1	R33590	regulator of G-protein signalling 19 interacting protein 1	–3.2	–4.1	–3.0	–1.5
	R09164		–2.4	–2.1	–1.4	–1.6
SMARCA5	R35122	SWI/SNF-related, matrix-associated, actin dependent regulator of chromatin, subfamily a, member 5	–1.7	–1.6	–1.4	–1.6
SMARCB1	AJ011737	SWI/SNF-related, matrix-associated, actin-dependent	–3.0	–1,8	–1.7	–1.2
	T97942	regulator of chromatin, subfamily b, member 1	–1.8	–1.3	–1.2	–1.6
STAT5B	T83012	signal transducer and activator of transcription 5B	–1.7 (–2.1)*	–1.4	–1.5	–1.2

Some genes were represented more than once in the array and values are given for each probe. Values for genes involved in the novel 0.5-Gy-pathway are italized. A list of relevant genes involved in the same pathway, but not specific for 0.5 Gy, is also included.
nd, not done.
* Values of quantitative RT-PCR.

PBLs [4]. Interestingly, over-expression of DDB2 in CD4+ T lymphocytes was observed in some donors 24 h after exposure to a very low dose of 0.01 Gy (data not shown).

In addition to known p53 targets, other genes were strongly activated (2 to 4 times up-regulated), such as the genes encoding ribosomal protein RPS27L, the RNA binding protein BRUNOL5 and the transcription factor Brn4/POU3F4, which deserve further consideration.

In contrast to CD4+ T cells, Jurkat cells showed only a modest increase in cell death after ionizing radiation, which was most visible after 48 h with 2 Gy.

We took advantage of the absence of the p53 pathway in Jurkat cells to unmask other pathways relevant to the cell type-specific responses to ionizing radiation. Of particular interest are genes modulated in Jurkat but not in CD4+ T cells (table 4). They include several genes that are induced in various stress conditions, such as *ATF6* and *ATF1* [14, 15]. This cluster of genes was modulated in all four conditions tested in Jurkat cells, but no specific pathways were identified. Whether some of these genes can be considered as a molecular signature for irradiation is currently under investigation. Furthermore, as only three of these genes were also modulated in CD4+ T lymphocytes (see table 4), we are investigating whether some of these genes characterize the response to radiation of transformed leukocytes.

When Jurkat cells were irradiated with 0.5 Gy, a set of genes was clearly repressed as shown by microarray (table 5) and for several of them by RT-PCR. They can be classified into three functionally related families: Rho-related genes and genes involved in STAT or NF-κB signaling pathways.

Rho-related genes are members of a signaling pathway that activates e.g. nuclear factor of activated T cells (NFAT)-dependent transcription [16] and acts on cytoskeleton remodeling (cell adhesion, polarization and migration). Their importance in immune responses has only recently been discovered [17]. After irradiation, several genes of this pathway were down-regulated, including the Rho nucleotide exchange factor *ARHGEF1*, which mediates signaling by RhoA, genes encoding the GTPase RhoG, cytoskeleton proteins (actin) and proteins involved in cytoskeleton remodeling (calponin H2, SWI/SNF family proteins and adhesion-regulating molecules).

In the STAT signaling pathway, both *STAT5B* and *STAT6* were repressed. STAT5, which is activated by interleukin (IL)-2R, IL-7R and IL-15R, induces IRF-1 expression, which was also repressed in the tested experimental conditions. STAT6 instead is activated by IL-4R. All these receptors (IL-2R, IL-7R, IL-15R and IL-4R) share the IL2Rγ chain as coreceptor. Repression of these STAT genes could lead to reduced cytokine signaling. Interestingly, the expression of *STAT5B* was down-regulated in both CD4+ T lymphocytes and Jurkat cells.

In the signaling pathway involving NF-κB, the gene coding for the inhibitor protein NFKBIB (IKB) was down-regulated after irradiation, which could result in positive modulation of this transcription factor. NF-κB activation in the T cells requires the coupled signal from the T cell receptor (TCR) and CD28. After stimulation, the activated TCR and CD28 signal to downstream pathways including activation of Vav1 (via ZAP-70), PI3-K and Akt (activated in our settings by ionizing radiation). These signals converge at the IKK kinases which phosphorylate IKB_s thus allowing subsequent destruction of IKB_s and activation of NF-κB. In this way, NF-κB activation has consequences in terms of proliferation and differentiation [18].

Taken together, our findings point to a novel pathway triggered by relatively low doses of ionizing radiation, which is mediated by members of the Rho-family GTPases, NF-κB and STAT signaling pathways, and which may lead to changes in the cytoskeleton, cytokine signaling, proliferation, survival and differentiation. Interestingly, *ARHGEF1*, *calponin H2*, *GDI1*, *RGS14* and *STAT5B* were also down-regulated in CD4+ T lymphocytes. Whether Rho, STAT and NF-κB signaling pathways are indeed functionally impaired in Jurkat cells and in normal leukocytes after irradiation is the subject of our on-going efforts.

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