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Influence of a low background radiation environment on biochemical and biological responses in V79 cells

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Abstract We present the results of an experiment aimed at comparing the effects of different background radiation environments on metabolism and responses to γ-rays and cycloheximide of cultured mammalian cells. Chinese hamster V79 cells were maintained in exponential growth in parallel for up to 9 months at the Istituto Superiore di Sanità (ISS) and at the INFN-Gran Sasso underground Laboratory (LNGS) where exposure due to γ-rays and to radon was reduced by factors of about 70 and 25, respectively. After 9 months the cells grown at the LNGS (cumulative γ dose about 30 μGy, average radon concentration around 5 Bq/m^3), compared to the cells grown at the ISS (cumulative γ-ray dose about 2 mGy, average radon concentration around 120 Bq/m^3), exhibited i) a significant increase of the cell density at confluence, ii) a significantly higher capacity to scavenge organic and inorganic hydroperoxides but a reduced scavenging capacity towards superoxide anions and iii) an increase in both the basal *hprt* mutation frequency and sensitivity to the mutagenic effect of γ-rays. The cells grown at the LNGS also showed a greater apoptotic sensitivity starting at the third month of culture, that was no longer detected after 9 months. Overall, these data suggest a role of background ionizing radiation in determining an adaptive response, although they cannot be considered conclusive.

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Introduction

In spite of the many epidemiological and experimental studies carried out for more than one century, there is no firm scientific knowledge on the health effects of humans having been exposed to ionizing radiation at low dose and at a low dose-rate.

Epidemiological studies are indeed of low statistical power at low level exposures. To gain information on the health effects of occupational, environmental and medical diagnostic concern, it is therefore necessary to extrapolate the risks known from studies at high dose acute exposures to low levels. As from 1959 [1], the International Commission on Radiological Protection (ICRP) has adopted a linear no-threshold (LNT) relationship between dose and health effects which is still maintained for low level exposures [2]. However, it is widely recognized that this extrapolation requires a detailed understanding of the mechanisms by which radiation induces cancer and genetic disorders [3], so that the LNT model, although widely used as a pragmatic guideline [4], could be too simplistic.

The current view is that even at low doses the reaction of cells and tissues is more complex than previously assumed. Examples of phenomena that may have a role in determining the dose-response relationship are i) adaptive response, ii) genomic instability, iii) low-dose hypersensitivity and iv) the occurrence of bystander effects in cell populations (for reviews, see [5, 6, 7, 8]).

Adaptive responses, i.e. the possibility that a small dose of radiation or chemical mutagenic agents, can condition cells so as to induce resistance to subsequent exposure to moderate or high doses of the same agents, was reported for the first time in 1984 by Olivieri et al. [9]. It is an issue of particular interest since it opens up

the possibility that small doses of radiation may reduce either the natural incidence of cancers or the likelihood of excess cancers. This observation prompted the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) to review the matter some years ago [10]. However, in the framework of the literature data, there is scarce information on the biological response after chronic exposure, i.e., exposure to very low doses and low dose-rates, such as those given by the natural radiation background.

Evaluation of this point is important not only to help assess the risks related to variation in the natural background and to chronic occupational exposure, but also to better understand the role of natural radiation background in the evolution of life on earth.

The Gran Sasso National Laboratory (LNGS) of the Istituto Nazionale di Fisica Nucleare (INFN), located under the Gran Sasso d'Italia mountain, offers a unique opportunity to investigate whether a significant reduction in the background exposure can change the susceptibility to acute exposure to genotoxic agents. It is one of the most impressive underground laboratories for astroparticle physics in the world. It was excavated along the highway tunnel crossing the Gran Sasso Massif and consists of three large experimental halls. The cover of at least 1,400 m of rock gives an excellent shielding against cosmic rays and neutrons. As a matter of fact, cosmic rays are reduced by a factor of 10^6, as reported for instance in [11], and neutrons by a factor of 10^3 [12, 13].

A few years ago, an experiment was performed on yeast cells cultured at the Gran Sasso Laboratory indicating that cells grown in a low background environment are less protected from mutational damage induced by methyl-methane sulfonate than cells grown at the Genetic Laboratory of Rome University "La Sapienza", in a "normal" background radiation environment [14].

In the current paper, results are described of a new experiment aimed at extending the previous observations to cultured mammalian cells, considering a variety of biological end-points. For this purpose, Chinese hamster V79 cells were cultured in parallel at the Physics Laboratory of the Istituto Superiore di Sanità (ISS), in a "normal" background radiation environment, and at the LNGS, in a low background radiation environment, for up to 9 months. The biological end-points considered were cell growth, apoptosis induction, antioxidant enzyme activity, and mutation induction.

Materials and methods

Laboratory set up at the LNGS

A cell culture laboratory was set up in a single room building located in the underground laboratory. The incubator is shielded by a 10 cm thick iron box to further reduce the background radiation during cell growth. An air duct is connected to the building roof to continuously flush the room inside (150 m^3/h) in order to avoid accumulation of radon gas.

Dosimetry

Characterization of the laboratories at the ISS and at the LNGS was accomplished in terms of both γ-rays and radon concentration. The activity originating from low/medium energy γ-rays was measured with thermoluminescence dosimeters (TLD) and organic scintillators. Three sets of TLDs were placed inside the incubators. Each set accumulated the dose over three periods: 30, 67 and 148 days. At the ISS, the average value of the γ-ray dose rate was 287±30 nGy/h, or 6.9±0.7 μGy/day, while the average γ-ray dose received by the cells grown at the LNGS was 4.3±0.9 nGy/h (103±22 nGy/day).

The organic scintillator was also placed in the incubators, and the dose-rates measured were 357±16 nGy/h at the ISS and 5.1±0.9 nGy/h at the LNGS.

From these measurements, compatible within two standard deviations, it can be estimated that there is a reduction factor of about 70 in the γ-ray activity at the LNGS compared to that at the ISS.

The average radon concentration, measured in the LNGS by means of a radometer was around 5 Bq/m^3. It is interesting to note that in the absence of ventilation the radon concentration can rise to about 100 Bq/m^3 within a few hours. Measurements at the ISS gave an average Rn concentration of around 120 Bq/m^3, i.e. almost 25 times higher than that at the LNGS.

Culture conditions

Chinese hamster V79 cells were grown as a monolayer in Eagle's minimal essential medium supplemented with 1 mmol dm^{-3} glutamine, antibiotics and 10% fetal calf serum, and subcultured 3 times a week in order to maintain them in exponential growth. The cultures were grown in parallel at the ISS and at the LNGS for 5 months, followed by a 3-month interruption. During this period the cultures were kept frozen in liquid nitrogen, each one in the respective laboratory. The cultures were then resumed for 2 months followed by a second interruption when they were kept again in liquid nitrogen for 5 months. Finally, the cultures were resumed for the last 2 months. The culture interruptions were due to civil engineering works in the underground Laboratory at the LNGS. The quoted dose received by the cells refers to the entire period of exponential growth only, that amounts to 9 months (corresponding to about 540 generations). To minimize the sources of variability, all reagents and chemicals used throughout the entire experiment were from the same batches.

The assays for all the end-points considered were performed at the beginning of the experiment and after 3 and 9 months of culture.

Cell growth

To measure cell growth, approximately 5×10^4 cells/dish were seeded in 6- cm-diameter Petri dishes and cultured for 7 days. Twice every day cells from two dishes were trypsinized, suspended in fresh medium and counted using a Burker emocytometer.

Apoptosis assessment

Apoptosis was induced by cycloheximide (CHX), a protein synthesis inhibitor, and was measured by flow cytometry. Petri dishes were seeded with 3×10^5 cells/dish and 24 h later, incubated for 6 h in the presence of 0, 50, 75, 100 and 200 μg/ml CHX.

For the cytofluorimetric measurements, $2–4\times10^6$ cells were washed 3 times, suspended in 1 ml of PBS, added drop-wise to 10 ml of 70% ethanol and stored at 4°C for 2–5 days. Cells were then centrifuged, washed once and suspended in 1 ml of PBS. An equal volume of staining solution (0.2% Triton X, 0.002 mmol dm^{-3} EDTA, 0.2 mg/ml of propidium iodide in PBS) was added and the samples kept on ice for 30 min.

The presence of the hypodiploid peak (that represents the subpopulation of apoptotic cells containing DNA<2c) in the cytofluorimetric pattern was monitored using a FACScan flow cytometer (Becton and Dickinson), standardized using chicken erythrocyte nuclei. The regions identifying the normal cycling cells and the hypodiploid cells were gated using a dot-plot of side-scattering against fluorescence area of the control cells.

Epifluorescence analysis of apoptosis was performed by two different staining procedures.

For the Annexin V-FITC (Zymed Laboratories) staining [15], approximately 3×10^5 cells/ml were centrifuged at 3,000 rpm for 1 min, washed with PBS and incubated for 10 min at room temperature with 190 µl of binding buffer and 10 µl of Annexin V-FITC, according to the manufacturer's instructions. Samples were washed, further incubated with 190 µl of binding buffer, 10 µl of propidium iodide and examined under an epifluorescence microscope (Axioplan 2, Zeiss).

For Hoechst 33258 staining, cells grown on cover-slips placed in Petri dishes were washed with PBS and fixed in 4% paraformaldehyde for 30 min at 4°C. Floating cells were also collected and seeded on a slide. Both cover slips and slides were stained with Hoechst 33258 and examined under an epifluorescence microscope.

Determination of p53 and c-myc expression

Parallel experiments on the CHX-treated cells were performed to investigate the expression of proliferation and apoptosis-related genes.

After rinsing with 1 mol dm^{-3} PBS (pH 7.6) cells were scraped and lysed in buffer containing 20 mmol dm^{-3} Tris-HCl (pH 7.6), 10% glycerol, 50 mol dm^{-3} NaCl, 1 mol dm^{-3} phenylmethylsulfonylfluoride (PMSF), 5 mol dm^{-3} EDTA, 5 mol dm^{-3} EGTA, 1% Triton X-100, 2% SDS, 2 mol dm^{-3} sodium orthovanadate, 20 µg/ml pepstatin, 20 µg/ml leupeptin and 15 µg/ml aprotinin. Samples were centrifuged at 14,000 rpm for 25 min, the pellet was discarded and protein concentrations determined by the Lowry assay [16]. After solubilization in 2% SDS, 6.2 mmol dm^{-3} Tris-HCl (pH 6.8), 10% glycerol and 5% β-mercaptoethanol, proteins were denatured at 100°C for 3 min, then resolved by SDS-PAGE with 4% stacking and 10% running gels cast in a minigel apparatus (Bio-Rad), loading 60 µg of cell proteins per lane. The transfer of the proteins to nitrocellulose sheets was carried out in Towbin transfer buffer at 350 mA for 90 min at 20°C [17]. Non-specific binding was blocked with 5% no-fat dry milk powder in TTBS (50 mol dm^{-3} Tris-HCl pH 7.5, 150 mol dm^{-3} NaCl, 0.1% Tween-20) at 4°C overnight. The blots were incubated with the primary antibody mouse monoclonal anti-p53 (Zymed Laboratories) or rabbit polyclonal anti-c-myc (Santa Cruz Biotechnology) for 90 min at room temperature, washed several times in TTBS followed by incubation with the appropriate affinity-purified secondary alkaline phosphatase-conjugated antibodies (anti-mouse and anti-rabbit IgG-AP for p53 and c-myc, respectively, Zymed Laboratories) for 90 min at room temperature. Immunocomplexes were visualized with the BCIP/NBT kit (Bio-Rad) and positive bands were scanned in a densitometer and photographed. Quantitative evaluations of the bands were performed using the Phoretix program.

Cell extract preparation and enzymatic assays

Cells were harvested and suspended in 10 mol dm^{-3} PBS (pH 7.0), containing 1 mmol dm^{-3} Triton X-100 (for catalase and superoxide dismutase assays) or 1 mmol dm^{-3} dithiothreitol (for glutathione peroxidase, glutathione reductase and glutathione transferase assays) at a concentration of 10^7 cells/ml. Cell suspensions were then homogenized with a Potter-Elvehyem homogenizer and the resulting cell extracts used for enzymatic assays. All measurements were performed at 25°C using a Perkin Elmer spectrophotometer.

Glutathione transferase (GST; EC 2.5.1.18) activity was recorded at 340 nm by the method described by Habig and Jacoby [18]. The conjugation reaction of 1-chloro-2,4-dinitrobenzene to glutathione (GSH) was monitored following the increase of absorbance at 340 nm (ε_{340nm}=9.6 $mmol^{-1}$ dm^{-3} cm^{-1}). One unit of enzyme activity was defined as 1 µmol of GSH conjugated/min.

Glutathione peroxidase (GSH-Px; EC 1.11.1.9) activity was measured according to the method of Paglia and Valentine [19] and glutathione reductase (GSSG-Rx; EC1.6.4.27) activity was measured according to Di Ilio et al. [20]. The oxidation of NADPH was monitored following the decrease of absorbance at 340 nm (ε_{340nm}=6.22 $mmol^{-1}$ dm^{-3} cm^{-1}). One unit of enzyme activity was defined as 1 µmol of NADPH oxidized/min.

Catalase (EC1.11.1.6) activity was measured spectrophotometrically [21]. The decomposition of H_2O_2 was monitored continuously at 240 nm (ε_{240nm}=0.040 $mmol^{-1}$ dm^{-3} cm^{-1}). Catalase activity units were defined as 1 µmol of H_2O_2 decomposed/min.

Superoxide dismutase (SOD; EC1.15.1.1) activity was assayed by its ability to inhibit the autoxidation of epinephrine, determined by the increase in the absorbance at 480 nm [22]. An appropriate amount of extract was used to obtain about 50% inhibition of the epinephrine autoxidation. A standard curve with purified superoxide dismutase, was obtained by plotting the inverse values of the amount of enzyme used versus the percentage inhibition observed, and this standard curve was used to determine the amount of extract necessary for a 50% inhibition (1 unit of SOD activity). Protein concentration was determined with the biuret method using BSA as standard.

Mutation assay

For mutation experiments, about 8×10^6 cells/dose were irradiated as monolayer with ^{137}Cs γ-rays. Doses up to 6 Gy were used, and the dose-rate was 1.1 Gy min^{-1}, as determined by TLD dosimetry. After irradiation, the cells were washed, detached, counted and diluted. Then aliquots of the cell suspension were plated at the appropriate density for survival determination. The remaining cells were subcultured every 48 h at a concentration of 1.5×10^6/175 -cm^2 flask (for a total of 6×10^6 cells/dose-point) to allow the phenotypic expression of mutation at the hypoxanthine-guanine phosphoribosyl transferase (*hprt*) locus. On days 6, 8 and 10 after irradiation, 10 Petri dishes of 90 -mm diameter were seeded with 3×10^5 cells/dish for the determination of *hprt* mutants growing in 6-thioguanine (1 µg/ml) selective medium. At the same time, 200 cells/dish were plated in each of 4 Petri dishes of 60 -mm diameter to determine the cloning efficiency.

For each experiment, the number of mutants per viable cell was determined averaging the results obtained from days 6–10 after irradiation.

Results

Effect of the low background environment on cell growth, apoptosis induction and expression of c-myc and p53

Cell growth capability was evaluated on V79 cells cultured for 3 and 9 months at the LNGS and at the ISS. Growth curves were obtained starting from an initial density of 5×10^4 cells/dish. Cell counts were performed every 12 h for 1 week and the results are shown in Fig. 1A,B. No differences could be observed in the growth curves of cells maintained at the ISS for 3 and 9 months, and of those maintained at the LNGS for 3 months. In all these cases the plateau was reached at around 4×10^6 cells/dish. On the contrary, cells grown at

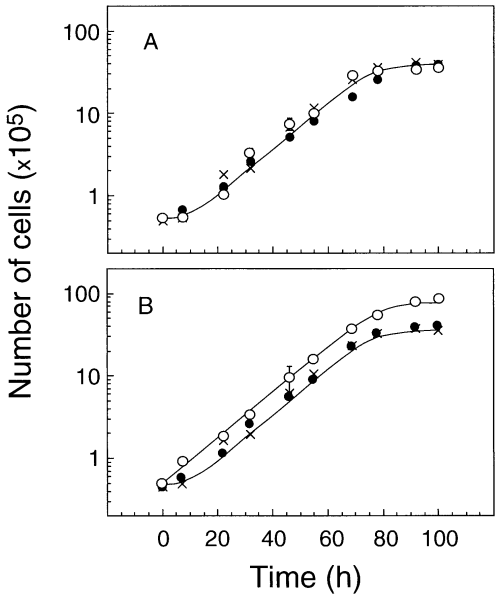

Fig. 1 Growth rate of V79 cells cultured at the ISS (*closed circle*) and at the LNGS (*open circle*), after 3 (*panel A*) and 9 (*panel B*) months of culture. Growth rate at zero time of culture (X) has been included in both panels**.** Data are the mean ±SE of at least 3 experiments, where cell counting was performed in duplicate

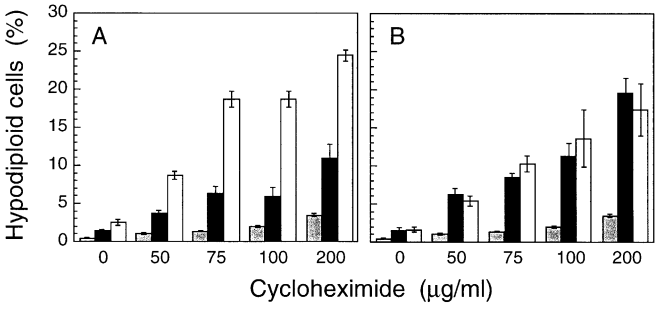

Fig. 2 Percentage of hypodiploid V79 cells as a function of cycloheximide (CHX) concentration. Cells after 3 (*panel A*) or 9 (*panel B*) months of culture at the ISS (*black bars*) or at the LNGS (*white bars*), were incubated for 6 h in the presence of CHX, fixed and analyzed by flow cytometry. Data obtained at zero time of culture (*gray bars*) have been included in both panels. Data are the mean±SE of at least 3 experiments

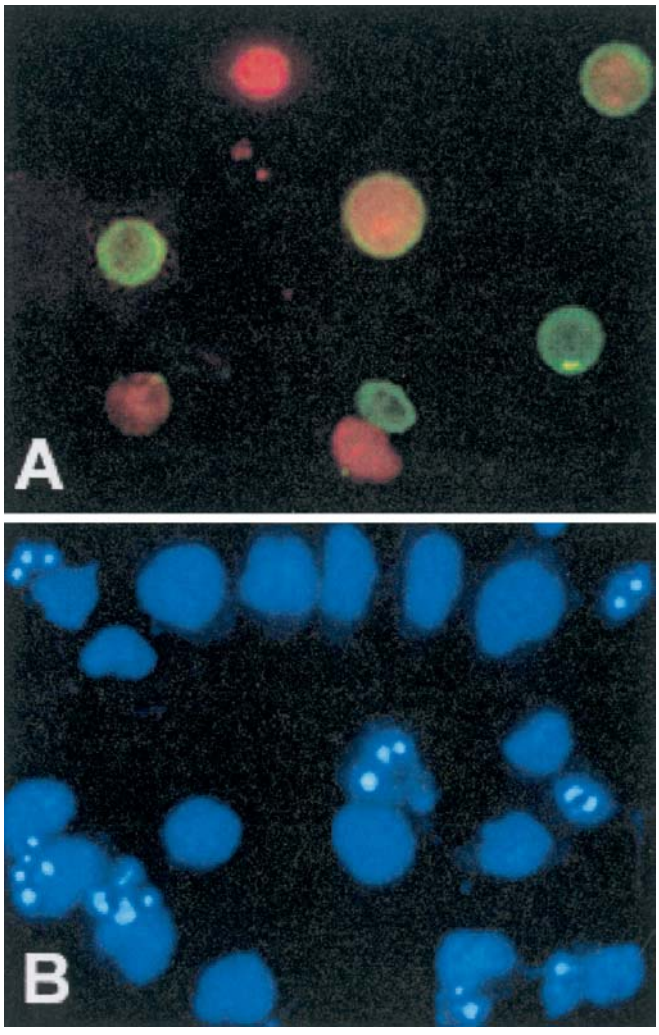

Fig. 3 *Panel A* V79 cell stained with Annexin V-FITC after 6 h of treatment with cycloheximide (CHX, 200 μg/ml). Cells in early phase of apoptosis are green stained on the cell membrane, cells in late phase of apoptosis are green stained on the cell membrane and red stained in the nucleus, the totally red stained are necrotic cells. *Panel B* Apoptotic cells stained with Hoechst 33258 after 6 h of treatment with CHX (200 μg/ml) showing the presence of fragmented nuclei. Magnification: 900×

the LNGS for 9 months reached the plateau at around 8×10^6 cells/dish, i.e. twice the concentration of the cells at the ISS.

Apoptosis induced by CHX was examined 6 h after drug addition, by evaluating the hypodiploid peak by cytofluorimetry, as described above. Both cell cultures showed a general increase in sensitivity to the drug as the culture time increased (Fig. 2A,B). When comparing cells at 3 months of culture, it appears that those grown at the LNGS were more sensitive to the induction of apoptosis by a factor of about 2, with respect to cells grown at the ISS. After 9 months, however, the sensitivity of both cell cultures was not significantly different.

The correspondence between the hypodiploid peak and apoptosis was confirmed by the morphological pattern obtained by Annexin V-FITC staining, a molecular membrane marker of apoptosis (Fig. 3A), and by Hoechst staining, indicating nuclear fragmentation (Fig. 3B) [23, 24].

Expression of c-myc and p53, two proteins well-known to be involved in both proliferation and apoptosis [25, 26, 27], was studied by Western blot analysis in cells grown at the ISS and at the LNGS (Fig. 4). Results show that the concentration of these two proteins strongly decreased after CHX treatment, apparently in a dose-independent way, and that there were no obvious differences between cells grown at the ISS or at the LNGS, neither at 3 months nor at 9 months. It is interesting to note that at 9 months, the basal levels of c-myc and p53 were differ-

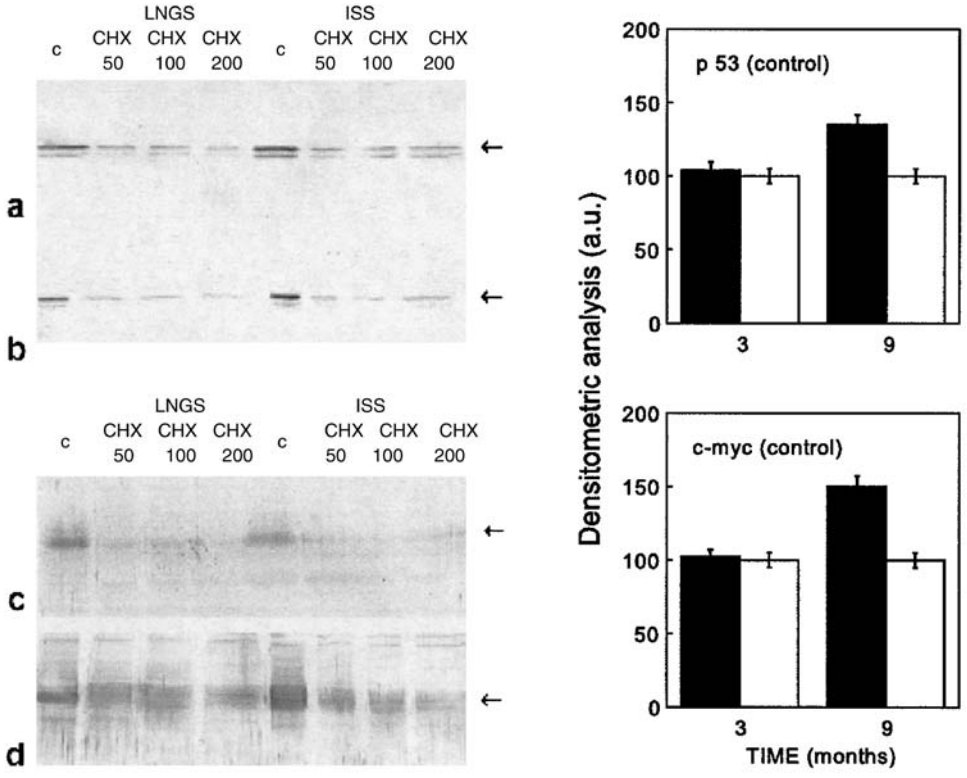

Fig. 4 Western blot of p53 (*upper panel*) and c-myc (*lower panel*) of V79 cells untreated **c**, and treated for 6 h with various concentrations of cycloheximide (CHX, μg/ml) at 3 **a, c** and 9 **b, d** months of culture. Densitometric evaluation of the bands of control cells is shown on the right: values are expressed as percentage of LNGS cells and represent the means of three separated experiments±SE. ISS (*black bars*), LNGS (*white bars*)

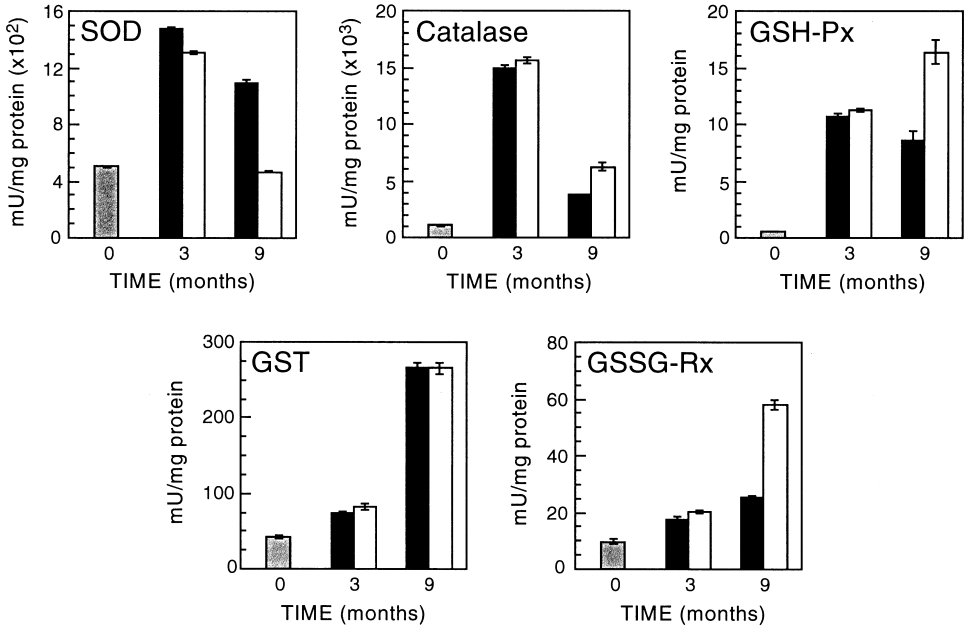

Fig. 5 Antioxidant enzyme specific activities in V79 cells at zero time (*gray bars*) and after 3 and 9 months of growth at the ISS (*black bars*) and at the LNGS (*white bars*). Activities are expressed as mU/mg protein and represent the mean ±SE of at least three different experiments where assays were performed in duplicate

ent in the two cell cultures. In fact, the expression of both proteins was higher in the ISS cells compared to the LNGS cells: the densitometric analysis gave band intensity ratios of 1.35 and 1.50 for p53 and c-myc, respectively.

Effect on antioxidant enzymatic activity pattern

Antioxidant enzymes were assayed in V79 cells after 3 and 9 months of culture. Figure 5 shows that after 3 months the specific activities of all the enzymes tested were significantly higher than those observed at zero time. After 9 months, this trend was maintained for GST, GSH-Px and GSSG-Rx. On the contrary, for SOD and catalase a significant decrease of the activities was observed with respect to 3 months, even if their values were still higher than at zero time.

When comparing cells grown at the ISS and at the LNGS, it appears that after 3 months only slight differences could be observed in the levels of all the enzymat-

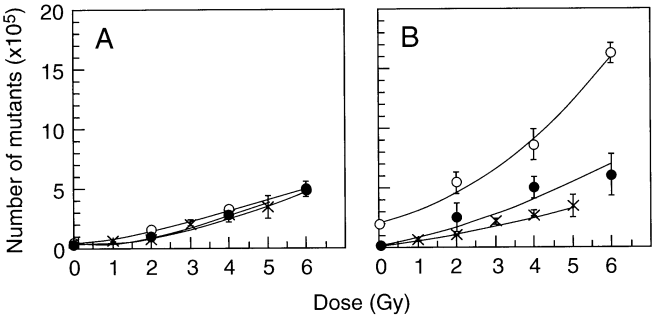

Fig. 6 Number of mutants as a function of different γ-ray dose. Cells grown for 3 (*panel A*) and 9 (*panel B*) months at the ISS (*closed circle*) and at the LNGS (*open circle*). Results obtained at zero time of culture (X) have been included in both panels. Each data point is the mean ±SE of 3 different determinations, performed on day 6, 8 and 10 after irradiation

ic activities. After 9 months, in the LNGS growing cells, SOD activity was lower than that of the ISS growing cells, while all the other enzymes showed significantly higher values, with the exception of GST activities which were similar.

Effect on mutation induction

For the cells grown at the LNGS, no significant differences in the background mutation frequency (bkMF) at the *hprt* locus were observed after 3 months with respect to zero time, while an increase from about $0.30\pm0.04\times10^{-5}$ to about $2.05\pm0.11\times10^{-5}$ was found after 9 months of culture.

On the contrary, the bkMF for the cells maintained at the ISS showed only small fluctuations, with a value of $0.46\pm0.06\times10^{-5}$ averaged over 9 months.

After γ-irradiation, no significant differences were observed in both cell cultures between the dose-response curves for mutation induction obtained at 0 and 3 months. On the contrary, after 9 months, a significant increase in the number of induced mutants was found in both cultures. Such increase was much more evident in cells grown at the LNGS (Fig. 6A,B) than in those grown at the ISS. In fact, after a dose of 6 Gy, the induced mutation frequency for the former was more than twice that for the latter.

Discussion and conclusions

To our knowledge, the experiment here described represents the first systematic study concerning the effects on mammalian cells, and for a variety of biological endpoints, of different levels of chronic exposures to ionizing radiation, such as those related to natural background.

With regard to cell growth, our results mainly show a significant increase in cell density at the plateau region of the growth curve after 9 months of culture at the LNGS. This effect could be related to modification in the molecular mechanisms involved in the control of contact inhibition. We did not observe any significant increase in the growth rate, in contrast to the results obtained many years ago for a protozoan and a cyanobacterium cultured in a low γ-radiation environment [28]. However, it is difficult to compare these results with ours, since they were obtained for simpler organisms and without control of the radon concentration.

In addition, at the third month of culture, the cells at the LNGS exhibited greater apoptotic sensitivity than those grown at the ISS. After such a time significant differences can no longer be detected between the two systems. This result could be explained considering that at 9 months the differences previously observed between cells grown at ISS and those at LNGS were masked by culture aging, which slows down cellular metabolic processes.

The observation that the increased apoptotic sensitivity, although dependent on aging, appeared earlier in LNGS cells than in ISS cells is consistent with the hypothesis of a role of the environmental radiation in the induction of defense mechanisms. The present data, although obtained after a long period of conditioning and not as a consequence of an acute treatment, are in agreement with several results both in vivo [29] and in vitro [30, 31] where the reduction of apoptotic rate following different stimuli was interpreted as an adaptive response that allows cells to acquire resistance to apoptosis induced by further damages.

The increase of the overall antioxidant enzymatic activities with culture time in both environmental conditions may be indicative of a cellular response to the increased concentration of reactive oxygen species (ROS), due to the aging process [32], through modulation of the expression of genes coding for antioxidant enzymes [33]. However, this modulation was different in the two experimental conditions, especially after 9 months, when cells grown at the LNGS, compared to those grown at the ISS, showed a strongly reduced scavenging capacity towards superoxide anions, whereas the capacity to scavenge organic and inorganic hydroperoxides appeared to be increased. While the SOD decrease observed in LNGS cells can be easily explained by considering that the production of superoxide anion, the natural SOD substrate, may be decreased in the low background radiation environment, thus down-regulating SOD expression, the significant increase in catalase, GSH-Px and GSSG-Rx activities in the LNGS cells is more difficult to interpret. However, the observation that the three enzymes show a similar behavior is quite consistent with the metabolically related functions. In fact, on the one hand catalase and GSH-Px are involved in the inorganic and organic hydroperoxide scavenging and on the other hand, GSG-Rx activity is strictly related to GSH-Px, since it supplies the latter with reduced glutathione. These results are consistent with the hypothesis that environmental radiation may quantitatively and qualitatively modulate the cellular metabolism, resulting in different expression of

antioxidant enzymes. Studies are in progress to assess possible different susceptibility of ISS and LNGS grown cells to oxidative stress stimuli.

Finally, the results concerning mutation induction show that after 9 months, both the basal *hprt* mutation frequency and cell sensitivity to the mutagenic effect of γ-rays are higher for LNGS than for ISS growing cells.

In conclusion, for all the examined biological end-points we have found differences associated with differences in the radiation background, even though in most cases it is not possible to exclude a superimposed effect due to culture aging. For some end-points this effect could be large enough to mask possible differences between the two cultures.

These data could be interpreted by the occurrence of an adaptive response due to variations of the natural background radiation. It is plausible that permanence of cells for a long time at reduced background can i) down-regulate expression of genes related to cell growth and repair, ii) modulate the antioxidant enzymatic pattern and iii) make cells more prone to misrepair of the radiation-induced DNA lesions.

However, it cannot be excluded that after many generations (9 months correspond to roughly 540 cell duplications), clones with different characteristics have been selected in the two cultures, independently of the different radiation background. Further experiments are needed to settle this issue.

Nonetheless, the data reported here are consistent with a role of environmental radiation in the response of a cell population to ionizing radiation, and suggest that this response may be more complex than that predicted by a linear relationship with the dose. This observation is not only important for a better understanding of the basic mechanisms in cell radiation biology, but also calls for further studies in order to ascertain possible implications for the estimation of risks to chronic exposures presently assumed for radiation protection purposes.

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