Role of DNA-dependent protein kinase in recognition of radiation-induced DNA damage in human peripheral blood mononuclear cells

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Abstract
The DNA-dependent protein kinase (DNA-PK) complex plays a crucial role in radiation-induced DNA damage recognition. The complex includes the ku heterodimer, which comprises ku 70 and ku 80 subunits, that binds DNA termini of breaks without sequence specificity, and the catalytic subunit DNA-PKcs. The activation of the DNA-PK complex was studied in X-irradiated peripheral blood mononuclear cells (PBMC) from subjects of different ages. Radiation-induced changes in the DNA-binding activity of the ku heterodimer, and in the concentrations of ku 70, ku 80, DNA-PKcs and phosphorylated ku 80 were determined in nuclear and cytoplasmic extracts. DNA-binding activity was increased by irradiation only in the nuclear extract of PBMC from young, but not from elderly subjects, whereas it was found unchanged in cytoplasmic extracts regardless of age. The radiation-induced activation of the DNA-PK complex may result from the increased concentrations of ku 80 and DNA-PKcs in the cytoplasm of PBMC from young, but not from elderly subjects, leading to a higher concentration of phosphorylated ku 80 which readily migrates to the nucleus where, after dimerization with ku 70, binds to DNA breaks. These findings suggest major steps involved in DNA-PK activation, and the intracellular and molecular changes that may account for the age-dependent impairment of DNA repair capacity in irradiated mammalian cells.

Introduction
The DNA-dependent protein kinase (DNA-PK) is a serine/threonine kinase consisting of a 470 kDa catalytic subunit (DNA-PKcs) and a heterodimeric regulatory complex, called ku, which is composed of 70 kDa (ku 70) and 86 kDa (ku 80) proteins (1). The ku heterodimer binds the ends of various types of DNA discontinuity, and is involved in the repair of DNA breaks caused by V(D)J recombination, isotype switching, physiological oxidation reactions, ionizing radiation and some chemotherapeutic drugs (1–6). The ku-dependent repair process, called non-homologous end joining, is the main DNA double-strand break repair mechanism in irradiated mammalian cells (7–9). The two subunits of the ku heterodimer associate tightly and may form a tetramer when bound to the two DNA ends of the break (10). Moreover, ku is able to translocate along DNA in an ATP-independent way (11,12). The ku heterodimer is probably involved in stabilizing broken DNA ends, bringing them together and preparing them for ligation (13), as well as in preventing digestion of the broken ends by DNA exonucleases (1). As ku has been described to activate mammalian DNA ligases in vitro (13), it is possible that ku and a ligase may be sufficient to repair breaks without further processing before ligation. After binding to the DNA ends, ku recruits DNA-PKcs to the breaks, activating its kinase function (14,15). Thus, ku acts as a subunit of DNA-PK and is largely responsible for the DNA-dependent activation of DNA-PKcs. The potential role of DNA-PKcs is to recruit other
repair components to the sites of breaks and regulate them by phosphorylation. Moreover, DNA-PKcs may signal the presence of DNA damage and induce cell cycle arrest or apoptosis (16), suggesting a role of this kinase in p53 activation. However, cells lacking DNA-PKcs or ku can still mediate a p53-dependent block of the cell cycle in response to ionizing radiation and other DNA-damaging agents (1), indicating that DNA-PKcs is only one of the several molecules that signal DNA damage.

In the present work, we investigated the effects of X-rays on the activation of the nuclear and cytoplasmic cascade of events leading to the recognition of DNA breaks by the DNA-PK complex. DNA breaks induced by the range of radiation doses used (2-5 Gy) have been well documented (17,18). Results herein also provide a molecular explanation for the age-related impairment of DNA repair in X-irradiated (19,20) mammalian cells.

Methods

Subjects

Subjects of different ages (12 young males, age ranging from 20 to 30 years, and 10 elderly males, age ranging from 75 to 88 years) were enrolled in this study. Heparinized samples were collected from peripheral blood. Samples were handled in separate experiments by the same group of researchers to avoid manipulation heterogeneity. The young subjects of this study were blood donors and were recruited by the Hematology Unit of the Catholic University of Rome. The elderly subjects were all resident in a public institution for retired people (Casa di Riposo Roma III) and monitored every 3 months. All subjects underwent the screening procedures outlined in the Senieur protocol (21) and classified as non-Senieur.

Irradiation procedure and cell stimulation

PBMC (~80% CD3+ cells, 5% CD19+ cells, 10% CD16+ cells, 5% monocytes) were collected by density-gradient centrifugation on Ficoll-Isopaque (Lymphoprep R; Nycomed, Asker, Norway) as described by Boyum (22) and washed twice with medium (RPMI 1640; Gibco, Grand Island, NY). A 5 ml aliquot of PBMC at the concentration of 10^6/ml was plated in culture dishes, irradiated with 2 or 5 Gy of X-rays, or left unirradiated. The X-ray machine (Gilandri, Lecco, Italy) was operated at 250 kV, 15 mA, 0.5 mm Cu filtration, dose rate 140 cGy/min in air, focus distance 50 cm. After irradiation, cells were recovered from culture dishes, washed with medium and resuspended in complete medium, consisting of RPMI 1640 supplemented with 10% FCS (Seromed, Berlin, Germany), 10 µg/ml gentamicin (EUROBIO, Les Ulis, France) and 2 mM L-glutamine (Flow, Paisley, UK). Cells (10×10^6/ml) were left unstimulated or they were stimulated with phytohemagglutinin (PHA; Gibco) at a final concentration of 1.2%. Cultures were incubated for 1 h, at 37°C, in a CO2 humidified incubator. At the end of the incubation, cells were lysed, and cytoplasmic and nuclear extracts were prepared.

Preparation of cytoplasmic and nuclear extracts

To this end, cells were firstly centrifuged at 350 g in a Beckman TJ-6 centrifuge.

To prepare cytoplasmic extracts, the pellet was resuspended in 0.5 ml of lysis buffer (HEPES 20 mM, pH 7.9, KCl 10 mM, EDTA 0.1 mM, DTT 1 mM, MgCl2 1.5 mM, PMSF 1 mM, NaF 200 mM and Na2P2O7 1 mM) and then kept on ice for 20 min. Subsequently, 25 µl of Nonidet P-40 (10%) was added and briefly vortexed. The homogenate was centrifuged at 12,000 g in a microfuge for 30 s at 4°C. The supernatant containing the cytoplasmic extract was removed and stored at −80°C. The pellet containing the nuclei was processed.
DNA-PK and DNA damage recognition in human PBMC

Fig. 3. Western blotting analysis of ku 70 and ku 80 in nuclear extracts of PBMC from a single young and a single elderly subject. Nuclear extracts were subjected to electrophoresis on a 7.5% polyacrylamide gel under reducing conditions. Proteins were then electrotransferred onto PVDF filters, and expression of ku 70 and ku 80 detected as described in Methods, by using the indicated antibodies. Anti-ku 70 was used as loading control for the nuclear extracts.

Fig. 4. Western blotting analysis of ku 70 and ku 80 in cytoplasmic extracts of PBMC from a single young and a single elderly subject. Cytoplasmic extracts were treated as in Fig. 3. Anti-β-actin was used as loading control for the cytoplasmic extracts. Vertical columns represent densitometric estimates of ku 80 as described in Methods.

Fig. 5. Western blotting analysis of DNA-PKcs in nuclear and cytoplasmic extracts of PBMC from a single young and a single elderly subject. Extracts were subjected to electrophoresis on a 5% polyacrylamide gel under reducing conditions. Proteins were then electrotransferred onto PVDF filters and expression of DNA-PKcs was detected as described in Methods, by using anti-DNA-PKcs antibodies. Vertical columns represent densitometric estimates as described in Methods.

Preparation of DNA probe

A double-stranded DNA fragment (56 bp) was prepared as follows: 10 pmol of the 56 oligomer (5’-GAT-CAG-TGA-TGG-AGT-TGG-CCA-CTC-CTC-TGG-GCG-CTC-GTC-GCA-CG-TCA-AGG-CC-3’) was end-labeled for 30 min at 37°C with T4 DNA polynucleotide kinase in the presence of 2 μl of [γ-32P]ATP (20 mCi/120 μl), then incubated with 20 pmol of the complementary oligonucleotide at 85°C for 5 min and subsequently cooled at room temperature. The probe was then purified with Micro BIO-Spin P-30 columns. The 56 oligomer sequence was constructed by modifying the sequence described (6) in order to obtain optimal ku binding. The DNA probe is widely used in gel electrophoreses performed under non-reducing conditions [electrophoretic mobility shift assay (EMSA)] to measure interactions between double-stranded DNA and proteins.

EMSA

A gel mobility shift assay to determine DNA end-binding activity of ku 70/80 was performed as follows. The radiolabeled DNA probe (1 ng: 100,000 c.p.m.) was incubated with 5 μl containing 2 μg of nuclear extract in the presence of 620 ng of circular plasmid pUC19 as unspecific competitor. The reaction was performed for 30 min at room temperature in 20 μl of binding buffer (Tris-HCl 10 mM, pH 7.5, EDTA 0.5 M, NaCl 150 mM, DTT 1 mM, PMSF 1 mM and glycerol 10%). The samples were electrophoresed in a 6% polyacrylamide gel at 200 V for 2 h at 4°C. The gel was fixed in 10% acetic acid, 10% methanol, dried on Whatman 3M and exposed to Amersham Pharmacia Biotech (Cologno Monzese, Italy) X-ray films overnight at -80°C. The supershift of the ku 70/80 heterodimer was performed by a rabbit anti-human ku 80 polyclonal antibody (AHP317; Serotec, Oxford, UK), as
DNA-PK and DNA damage recognition in human PBMC

For DNA-PKcs evaluation, boiled samples were subjected to SDS–PAGE using a 5% polyacrylamide gel. The polyclonal rabbit anti-human DNA-PKcs antibody (AHP318; Serotec) was used as primary antibody. Following 1 h incubation with the primary antibody, immunoblots were developed by using an anti-rabbit alkaline phosphatase-conjugated secondary antibody and the ECF (Amersham) substrate was used to develop the reaction (chemifluorescence). Fluorescence was acquired by the phosphoimager/fluorimager STORM840 (Molecular Dynamics, Sunnyvale, CA) and the intensity of the bands was directly quantified by Image QuaNT software (Molecular Dynamics) which gives rise to a volume report by integrating the area of the band and its density.

Fig. 6. Western blotting analysis of phosphorylated ku 80 in nuclear and cytoplasmic extracts of PBMC from a single young and a single elderly subject. Immunoprecipitation was conducted by incubating crude cytoplasmic and nuclear extracts with anti-ku 80 antibody for 2 h at 4°C in agitation. After this time, Protein A–Sepharose was added and incubated for 2 h at 4°C in agitation. Immunoprecipitates were collected, resuspended in a denaturating solution and then boiled for 5–7 min. After protein content determination, immunoprecipitated ku 80 was split into two aliquots: one was run in Western blotting in the presence of anti-phosphoserine/threonine antibody, whereas the other one in the presence of anti-ku 80 antibody. Following 1 h incubation with the primary antibody, immunoblots were developed as described. Vertical columns represent densitometric estimates as described in Methods.

Fig. 7. Phosphorylation of ku 80 by DNA-PKcs in the cytoplasm of irradiated PBMC. ku 80 and DNA-PKcs were both immunoprecipitated from cytoplasmic extracts and run in a Western blotting, alone or mixed together, in the presence of anti-phosphoserine/threonine antibody. The ratio DNA-PKcs:ku 80 was 1:2. The Western blotting was performed in the presence of 10 mM ATP.

**Results**

DNA-binding activity of the ku 70/80 heterodimer in nuclear and cytoplasmic extracts of PBMC

PBMC were irradiated with 2 or 5 Gy and, immediately after X-ray exposure, were PHA-stimulated for 1 h. Under these conditions, preliminary results (not shown) have demonstrated maximal enhancement of DNA-binding activity of ku from irradiated PBMC as compared to unirradiated controls. After PHA stimulation, nuclear and cytoplasmic extracts were prepared and tested by EMSA. DNA-binding activity of ku in nuclear extracts of unirradiated or 2 or 5 Gy-treated PBMC from young and elderly subjects is shown in Fig. 1. DNA binding of nuclear ku is unchanged by ageing and is increased by 2 or 5 Gy X-irradiation of PBMC from young, but not from elderly subjects. The DNA-binding activity of ku is likely to described (20). The antibody was used according to the manufacturer’s protocol.

**Western blotting**

For the evaluation of ku 70 and ku 80 amounts in the cytoplasm and in the nucleus of PBMC, cytoplasmic and nuclear extracts at equal protein concentration were denatured by boiling for 5 min in Laemli sample buffer, and subjected to SDS–PAGE using a 7.5% polyacrylamide gel under reducing conditions. Proteins were then electrotransferred onto PVDF filters (Amersham) at 30 V overnight at 4°C. Non-specific sites were blocked by incubation of the membranes with 20 mM Tris–HCl (pH 7.4), 137 mM NaCl and 0.1% Tween-20 containing 5% BSA, for 1 h at room temperature (blocking solution). Filters were incubated with a rabbit anti-human ku 70 and anti-ku 80 polyclonal antibodies (AHP316 and AHP317 respectively; Serotec). Anti-ku 70 polyclonal antibody and anti-β-actin mAb (clone AC-15, A5441; Sigma, St Louis, MO) were used as loading controls for the nuclear and cytoplasmic extracts respectively.

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The evaluation of the amount of phosphorylated ku 80 in the cytoplasm and in the nucleus of PBMC was performed on immunoprecipitated samples. After protein determination, immunoprecipitation was conducted by incubating crude cytoplasmic and nuclear extracts (600 µg in 200 µl) with 2 µl of anti-ku 80 antibody for 2 h at 4°C in agitation. After this time, Protein A–Sepharose (Pharmacia) was added (20 µl) and incubated for 2 h at 4°C in agitation. Immunoprecipitates were collected by pelleting at 13,000 r.p.m. in a microfuge and resuspended in 10 µl of a denaturating solution (10% SDS and 4.5% β-mercaptoethanol 5×10⁻⁵ M). Immunoprecipitates were then boiled for 5–7 min and protein content determined. Immunoprecipitated ku 80 was split into two aliquots: one was determined by Western blotting in the presence of anti-phosphoserine/threonine (P3430; Sigma) antibody, whereas the other one in the presence of anti-ku 80 antibody. Following 1 h incubation with the primary antibody, immunoblots were developed as described above.

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Table 1. Summary of ku 80, phosphorylated (Ph)-ku 80 and DNA-PKcs densitometric estimates

<table>
<thead>
<tr>
<th>Estimated parameters</th>
<th>Cytoplasm</th>
<th>Nucleus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unirradiated</td>
<td>Irradiated</td>
</tr>
<tr>
<td>ku 80</td>
<td>7.8 ± 0.2</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>DNA-PKcs</td>
<td>17.9 ± 0.6</td>
<td>0.4 ± 0.3</td>
</tr>
<tr>
<td>Ph-ku 80</td>
<td>8.3 ± 0.3</td>
<td>9.1 ± 0.1</td>
</tr>
</tbody>
</table>

Mean values ± SE.

Comparisons of ku 80 are allowed within the cytoplasm or nucleus but not between cytoplasm and nucleus, because, unlike DNA-PKcs and Ph-ku 80, cytoplasmic and nuclear extracts were run on separate gels.

Two-tailed Student’s t-test was carried out to evaluate the statistical significance of the difference between the following means:

- ku 80 in young cytoplasm, unirradiated versus irradiated: P < 0.01.
- ku 80 in elderly cytoplasm, unirradiated versus irradiated: P < 0.01.
- ku 80 in young nucleus, unirradiated versus irradiated: not significant.
- ku 80 in elderly nucleus, unirradiated versus irradiated: not significant.
- DNA-PKcs in young cytoplasm, unirradiated versus irradiated: P < 0.05.
- DNA-PKcs in elderly cytoplasm, unirradiated versus irradiated: not significant.
- DNA-PKcs in young nucleus, unirradiated versus irradiated: not significant.
- DNA-PKcs in elderly nucleus, unirradiated versus irradiated: not significant.
- Ph-ku 80 in young cytoplasm, unirradiated versus irradiated: P < 0.05.
- Ph-ku 80 in elderly cytoplasm, unirradiated versus irradiated: not significant.
- Ph-ku 80 in young nucleus, unirradiated versus irradiated: not significant.
- Ph-ku 80 in elderly nucleus, unirradiated versus irradiated: not significant.

Reflect the binding of multiple copies of ku to the same DNA probe, as already observed (20). In the experiments reported in Fig. 1, the polyclonal rabbit antibody, specific for the human ku 80 protein, was able to supershift the band due to the DNA-binding of ku.

Figure 2 refers to DNA-binding activity of ku in cytoplasmic extracts from the same subjects of Fig. 1. The cytoplasm of unirradiated PBMC from an elderly, but not from a young subject, exhibits DNA binding of the ku heterodimer. This cytoplasmic activity is not affected by irradiation of PBMC from the young and elderly subjects. In the experiment of Fig. 2, the supershift of the bands due to the DNA binding of ku is also reported.

Nuclear and cytoplasmic expression of ku 70 and ku 80

Since in a young subject only the nuclear ku binds DNA, whereas in an elderly subject both nuclear and cytoplasmic ku bind DNA, we examined the levels of ku 70 and ku 80 proteins by Western blotting in nuclear and cytoplasmic extracts from unirradiated or 2–5 Gy X-ray-treated PBMC. Results in Fig. 3 show that X-rays do not affect the amount of ku 70 and ku 80 in nuclear extracts of PBMC from both young and elderly subjects.

Figure 4 shows that ageing does not modify the amount of ku 70 in the cytoplasm, but significantly reduces the amount of ku 80. Moreover, in the cytoplasm of PBMC from a young subject, the amount of ku 70 protein is unchanged by X-rays, whereas that of ku 80 is 2-fold increased after 2 and 5 Gy. In the elderly, ku 70 is not modified by X-rays, whereas ku 80 is up-regulated by irradiation (4- and 3-fold after 2 and 5 Gy respectively) as compared to the unirradiated control. It is noteworthy that the small amount of ku 80 protein in cytoplasmic extracts of PBMC from the elderly subject is able, together with ku 70, to bind DNA.

Nuclear and cytoplasmic expression of DNA-PKcs

The role of ku is to provide the DNA-binding activity of the DNA-PK complex by joining the catalytic subunit DNA-PKcs, an association that cannot be detected under the salt conditions used in EMSA. Therefore, the levels of DNA-PKcs in nuclear and cytoplasmic extracts of PBMC from young and elderly subjects were investigated by Western blotting. Results in Fig. 5 show that the amount of DNA-PKcs in nuclear extracts of PBMC from young and elderly subjects is not modiﬁed by ageing. Moreover, in the young subject the amount of DNA-PKcs in the nucleus is unaffected by irradiation, whereas in the cytoplasm is signiﬁcantly increased by 5 but not 2 Gy irradiation. In the elderly, DNA-PKcs is present in the nucleus, where it is not affected by irradiation, but not in the cytoplasm. The increased amount of DNA-PKcs in the cytoplasm of irradiated PBMC from the young subject suggests that this kinase activates ku 80 by phosphorylation in the cytoplasm from which the phosphorylated molecule then may translocate into the nucleus.

Phosphorylation of ku 80 in the cytoplasm and in the nucleus

Evaluation of the amount of phosphorylated ku 80 in the cytoplasm and in the nucleus of normal or 5 Gy-treated PBMC from a young or an elderly subject is reported in Fig. 6. Results show that the amount of phosphorylated ku 80 is signiﬁcantly decreased in the cytoplasm but increased in the nucleus of irradiated PBMC from the young subject. At variance, in PBMC from the elderly subject no changes in the amount of phosphorylated ku 80 were induced by irradiation in both nucleus and cytoplasm. These results support the possibility that the catalytic subunit of the DNA-PK complex phosphorylates ku 80 in the cytoplasm from which the phosphorylated molecule then translocates to the nucleus where it recognizes radiation-induced DNA damage. The hypothesis...
that ku 80 is phosphorylated by DNA-PKcs in the cytoplasm is also supported by the observation, shown in Fig. 7, that mixing ku 80 with DNA-PKcs, both immunoprecipitated from cytoplasmic extracts, yields ku 80 detectable in Western blotting by anti-phosphoserine/threonine antibody.

The findings shown in all figures refer to single young and elderly subjects. The major Western blotting results are summarized in Table 1 as densitometric mean values representing the estimates in cytoplasmic and nuclear extracts of PBMC from the young and elderly subjects investigated.

**Discussion**

The recruitment and activation mechanisms of the molecules involved in DNA damage recognition and repair after cell exposure to genotoxic stress are poorly understood. The DNA-PK complex is considered to play a crucial role, because it is implicated in three of the four complementation groups of mammalian cell mutants specifically defective in double-strand break repair (4,15). In the present paper, we describe the involvement of the DNA-PK complex in response to X-rays in PBMC from subjects of different ages. Results herein demonstrate that the ku heterodimer, which represents the DNA-targeting component of the serine/threonine kinase DNA-PKcs (23), maintains its ability to bind DNA in unirradiated cells during ageing. This is a relevant observation as ku has been proposed not only to activate DNA-PKcs, but also to exert several other functions such as protection of DNA ends from degradation and dissociation of the recombination-activating protein RAG–DNA complex to facilitate the joining reaction (1). However, the radiation-induced activation of ku, in terms of DNA binding, is different in PBMC from young and elderly subjects, as ku displays increased activity in nuclear extracts of irradiated PBMC from young but not from elderly subjects.

Experiments were performed with doses (2 or 5 Gy) of X-rays which generate a modest amount of DNA breaks (17,18) with high probability of an accurate repair. With higher radiation doses, the DNA fragmentation is increased with greater probability of rejoining unrelated ends and leading to mismatched repair and apoptosis.

It should be pointed out that, unlike previous studies carried out on whole lysates of cell lines, the present investigation was performed on nuclear and cytoplasmic extracts of freshly isolated cells to dissect out the intracellular and molecular steps leading to DNA-PK activation in vivo. DNA radiation damage in PBMC from young subjects appears to induce increased amounts of ku 80 and DNA-PKcs in the cytoplasm, probably resulting from enhancement of their syntheses. Furthermore, this kinase activates ku 80 by phosphorylation in the cytoplasm from which the activated ku 80 readily translocates to the nucleus with concomitant decrease of its concentration in the cytoplasm. It follows that DNA-binding activity of the ku heterodimer is detected only in the nucleus. The hypothesis that ku 80 is phosphorylated by DNA-PKcs in the cytoplasm is strongly sustained by the results of Fig. 7. The findings in cytoplasmic and nuclear extracts of PBMC from young subjects are in line with previous observations demonstrating that ku is in vitro phosphorylated by DNA-PKcs (24) and that cells lacking DNA-PKcs are deficient in phosphorylated ku (25), suggesting that ku is a physiologic target of DNA-PKcs activity in vivo (26). Although it has been demonstrated that ku recruits DNA-PKcs to DNA breaks and activates its kinase function, there is evidence that DNA-PKcs binds to linear DNA fragments in vitro and becomes activated for kinase activity in the absence of ku (27). This possibility is not excluded by the present results.

In the elderly, the radiation-induced activation of DNA-binding activity of nuclear ku is undetectable. In the cytoplasm, ku 80, but not ku 70, is significantly increased by irradiation. DNA-PKcs is present only in the nucleus but not in the cytoplasm, suggesting that in the elderly the total cellular content of this kinase is reduced and localized in the nucleus. Moreover, the amount of phosphorylated ku 80 is unchanged in nuclear extracts of irradiated PBMC from elderly subjects, as compared to unirradiated controls. Thus, in the elderly, the reduced amount of DNA-PKcs may account for the low amount of phosphorylated ku 80. Furthermore, the radiation-induced translocation of phosphorylated ku from the cytoplasm to the nucleus may also be defective in the elderly, as suggested by the DNA-binding activity retained in the cytoplasm.

Recent observations (28–30) have suggested that DNA-PK plays a pivotal role in apoptosis. The cell cycle arrest induced by DNA damage may result in DNA repair and therefore in removal of the cell cycle blockade, when the repair is completed. If, however, cells undergo apoptosis their capacity to repair damaged DNA is suppressed, also owing to the cleavage of repair enzymes by proteases (29). The threshold of DNA damage leading to repair or apoptosis is strictly dependent on the self-renewal capacity of the cell. Cells from young subjects have elevated self-renewal capacity and, therefore, are likely to exhibit a higher threshold for radiation-induced apoptosis and increased DNA repair potential, as compared to cells from elderly subjects. An increased susceptibility of cells from elderly subjects to anti-Fas-induced (31) and to tumor necrosis factor-a-induced apoptosis (32) has indeed been described. However, since dysregulation of several molecules that control apoptosis has also been reported in ageing (30), cells with reduced apoptosis can escape from this deletion process, undergo proliferation and mutate their phenotype to display tumor markers. Further work is needed to better understand the link between DNA repair and apoptosis in the response of PBMC from subjects of different ages to radiation-induced DNA damage.

**Abbreviations**

- DNA-PK: DNA-dependent protein kinase
- EMSA: electrophoretic mobility shift assay
- PBMC: peripheral blood mononuclear cells
- PHA: phytohemagglutinin

**References**