

## Gamma-radiation-induced ATM-dependent signalling in human T-lymphocyte leukemic cells, MOLT-4

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ATM kinase (ATM) is essential for activation of cell cycle check points and DNA repair in response to ionizing radiation (IR). In this work we studied the molecular mechanisms regulating DNA repair and cell death in human T-lymphocyte leukemic cells, MOLT-4. Apoptosis was evaluated by flow-cytometric detection of annexin V. Early apoptotic cells were determined as sub-G1 cells and late apoptotic cells were determined as APO2.7-positive ones. Proteins involved in ATM signalling pathway were analysed by Western-blotting. We observed a rapid (0.5 h) phosphorylation of ATM declining after 6 h after irradiation by all the doses studied (1.5, 3.0, and 7.5 Gy). Checkpoint kinase-2 (Chk-2) was also phosphorylated after 0.5 h but its phosphorylated form persisted 4, 2, and 1 h after the doses of 1.5, 3.0, and 7.5 Gy, respectively. The amount of p53 protein and its form phosphorylated on Ser-392 increased 1 h after irradiation (1–10 Gy). The lethal dose of 7.5 Gy caused an immediate induction and phosphorylation of p53 after 0.5 h post-irradiation. At the time of phosphorylation of p53, we found simultaneous phosphorylation of the oncoprotein Mdm2 on Ser-166. Neither ATM nor its downstream targets showed a dose-dependent response after 1 h when irradiated by the doses of 1–10 Gy. MOLT-4 cells were very sensitive to the effect of IR. Even low doses, such as 1.5 Gy, induced apoptosis 16 h after irradiation (evaluated according to the cleavage of nuclear lamin B to a 48-kDa fragment). IR-induced molecular signalling after exposure to all the tested doses was triggered by rapid phosphorylation of ATM and Chk-2. Subsequent induction of p53 protein and its phosphorylation was accompanied by concomitant phosphorylation of its negative regulator, oncoprotein Mdm2, and followed by induction of apoptosis.

**Keywords:** ATM kinase, checkpoint kinase-2, p53, Mdm2, ionizing radiation

### INTRODUCTION

Various physical and chemical factors induce DNA lesions, including base damage, intra- and inter-strand cross-linking, and single- and double-strand breaks (DSBs) (Khanna *et al.*, 2001). DSBs cause remodelling of chromatin and formation of

so-called ionizing radiation (IR)-induced foci, where the proteins involved in radiation damage repair are localized within the first minutes after irradiation (Bekker-Jensen *et al.*, 2006). A very early step in the response of mammalian cells to DSBs induced by IR is activation of ATM kinase (Lavin *et al.*, 1995). ATM stands for ataxia-telangiectasia (A-T, a human auto-

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**Abbreviations:** ATM, ataxia telangiectasia-mutated kinase; Chk-2, checkpoint kinase-2; DSB, double strand break; IR, ionizing radiation; Mdm2, murine double minute 2 protein; wt, wild type.

somal recessive disorder), whose responsible gene is mutated. *ATM*, the defective gene in this pleiotropic disease (progressive cerebral ataxia, oculocutaneous telangiectasia, immunodeficiency), encodes a large serine/threonine kinase belonging to the phosphatidylinositol-3 kinase family (Powers *et al.*, 2004). A-T cells exhibit a specific phenotype including increased radiosensitivity, genome instability, cancer predispositions and others (Lavin & Shiloh, 1997). Cells derived from A-T patients are extremely sensitive to IR, radiomimetic drugs and topoisomerase inhibitors. The importance of ATM is undeniable, since it regulates all three cell cycle checkpoints and functions in the regulation of DNA repair and apoptosis, suggesting that it is a central controller of cellular responses to DSBs (Khanna *et al.*, 2001). Activation of ATM involves rapid intermolecular autophosphorylation of Ser-1981 that causes dissociation of the inactive dimer (Bakkenist & Kastan, 2003). ATM affects numerous different targets *via* phosphorylation and some of them participate in the cell cycle arrest. These are p53, murine-double minute protein (Mdm2), and checkpoint kinase-2 (Chk-2) in the G1 checkpoint (Canman *et al.*, 1998; Matsuoka *et al.*, 2000; Maya *et al.*, 2001); Nbs1, Brca1, FancD2, and SMC1 in the transient IR-induced S-phase arrest (Taniguchi *et al.*, 2002; Yazdi *et al.*, 2002; Xu *et al.*, 2002); and Brca1 and hRad17 in the G2/M checkpoint (Xu *et al.*, 2001; Bao *et al.*, 2001).

In this work we studied the molecular pathway initiated by IR in MOLT-4 cells. These cells are very radiosensitive; by clonogenic survival assay we determined the  $D_0$  value (the dose reducing cell survival to 37%) at 0.87 Gy, whereas for the human promyelocyte leukemic cell line HL-60 the  $D_0$  is 2.2 Gy (Vavrova *et al.*, 2004). We investigated mechanisms related to phosphorylation of ATM (Ser-1981) and therefore we studied the phosphorylation of p53 (Ser-392), Chk-2 (Thr-68) and Mdm2 (Ser-166). Here, we report a rapid phosphorylation of ATM and Chk-2 after exposure of cells to IR. We found that protein p53 was induced shortly after activation of ATM and Chk-2. Subsequent phosphorylation of p53 on Ser-392 appeared soon after induction of p53, facilitating its ability to bind to DNA. That was accompanied by concurrent phosphorylation of Mdm2 on Ser-166, which provides a platform for p53 degradation and decreases its transcriptional activity as part of an auto-regulatory feedback loop.

## METHODS

**Cell cultures and culture conditions.** MOLT-4 cells were obtained from the American Type Culture Collections (Manassas, VA, USA). The cells were cultured in Iscove's modified Dulbecco's medium (Sig-

ma) supplemented with 20% fetal calf serum, 0.05% L-glutamine, 150 UI/ml penicillin, 50 µg/ml streptomycin in a humidified atmosphere with 5% CO<sub>2</sub> at 37°C. The cultures were split every second day by dilution to a concentration of  $2 \times 10^5$  cells/ml. The cell counts were performed with a hemocytometer; the cell membrane integrity was determined by using the Trypan blue exclusion technique. Cell lines at up to 20 passages were used for this study.

**Gamma irradiation.** Exponentially growing MOLT-4 cells were suspended at a concentration of  $2 \times 10^5$ /ml. Aliquots of 10 ml of cell suspension were plated into 25 cm<sup>2</sup> flasks (Nunc) and irradiated at room temperature using <sup>60</sup>Co gamma-ray source with a dose-rate of 0.4 Gy/min, at a distance of 1 m from the source. After the irradiation the flasks were placed in a 37°C incubator with 5% CO<sub>2</sub> and aliquots of the cells were removed at various times after irradiation for analysis. The cells were counted and cell viability was determined with the Trypan blue exclusion assay.

**Flow-cytometric apoptosis detection.** We used APOPTTEST-FITC (DakoCytomation, Brno, CZ) for detection of apoptotic cells (annexin V-positive) according to the manufacturer's guide. Analysis was performed on an Epics XL flow-cytometer (Coulter Electronic, Hialeah, FL, USA).

Early apoptotic cells were determined as sub-G1 cells in DNA content analysis. Following the incubation,  $1 \times 10^4$  cells were washed with cold PBS (phosphate-buffered saline), fixed with 70% ethanol and stained with propidium iodide in Vindelov's solution for 30 min at 37°C. Fluorescence (DNA content) was measured using the Coulter Electronic apparatus.

Late apoptotic cells were determined by detection of the APO2.7 antigen. The cells were collected and washed twice with cold PBS with 5% fetal calf serum. Subsequently,  $1 \times 10^5$  of cells were resuspended in 0.5 ml of PBS with 5% fetal calf serum and incubated with mouse monoclonal anti-APO2.7 antibody conjugated with phycoerythrin (Beckman Coulter, Fullerton, CA, USA) for 0.5 h at 4°C and measured using the Coulter Electronic apparatus.

In all experiments three independent measurements were performed. Data were analysed using Epics XL System II software (Coulter Electronic).

**Electrophoresis and Western blotting.** At various times after irradiation, the MOLT-4 cells were washed with PBS and lysed. Whole cell extracts were prepared by lysis in 500 µl of lysis buffer (137 mM NaCl, 10% glycerol, 1% *n*-octyl-β-glucopyranoside, 50 mM NaF, 20 mM Tris/HCl, pH=8, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 tablet of protein inhibitors Complete™ Mini, Roche). The lysates containing equal amounts of protein (30 µg) were loaded onto a 12% SDS/polyacrylamide gel. After electrophore-

sis, proteins were transferred to a PVDF membrane and hybridized with an appropriate antibody (anti-ATM (Ser-1981), anti-Chk-2 (Thr-68) or anti-Mdm2 (Ser-166) from Upstate, Chicago, IL, USA; anti-p53 or anti-p53 (Ser-392) from Exbio, Prague, CZ; anti-lamin B from Oncogene, Cambridge, MA, USA). After washing, the blots were incubated with a secondary peroxidase-conjugated antibody (Dako, High Wycombe, UK) and the signal was developed with a chemiluminescence (ECL) detection kit (Boehringer Mannheim) by exposure to an X-ray film (Foma, Hradec Kralove, CZ).

## RESULTS

### Induction of apoptosis

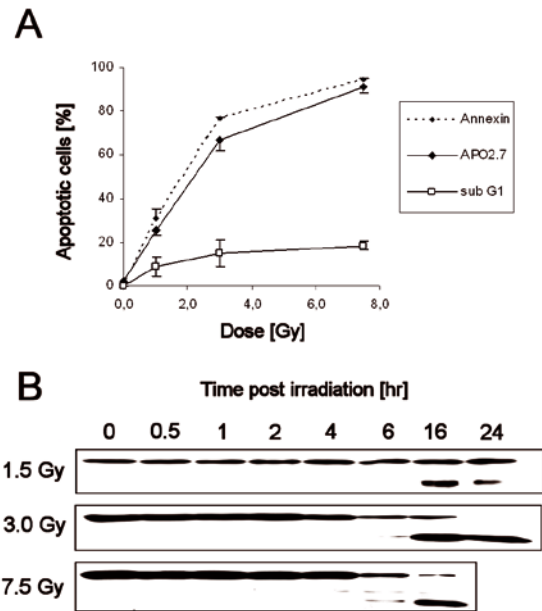
For detection of apoptosis 24 h after the irradiation we used four different methods: annexin V binding, DNA content measurement, detection of 7A6 antigen and caspase-catalysed cleavage of lamin B.

The first method uses flow-cytometric detection of annexin V binding to phosphatidylserine. Exposure of this phospholipid at the plasma membrane is a hallmark of apoptotic process; the method detects both early and late apoptotic cells. After 24 h, 30.7, 76.9, and 94.4% of cells were apoptotic when irradiated by 1.5, 3.0, and 7.5 Gy, respectively.

For determination of early apoptosis we used flow-cytometric determination of DNA content and so-called sub-G1 peak corresponding to apoptotic cells. After 24 h, 8.8, 15.1 and 18.4% of cells were apoptotic when irradiated by 1.5, 3.0, and 7.5 Gy, respectively.

For determination of late apoptosis we used flow-cytometric detection of APO2.7 antigen. Since this antigen is localized on the mitochondrial membrane of apoptotic cells, it is possible to perform the assay with permeabilization or without permeabilization, to detect the cells whose plasma membrane is already permeable. To determine cells in the late stage of apoptosis the method without permeabilization was used in this study. After 24 h, 25.5, 66.7, and 91.0% of cells were apoptotic when irradiated by 1.5, 3.0, and 7.5 Gy, respectively. Figure 1A shows the percentage of annexin V-positive, APO2.7-positive and sub-G1 cells 24 h after gamma irradiation with increasing doses.

Another method confirming the induction of apoptosis of MOLT-4 cells was Western blot analysis of fragmented nuclear protein lamin B. In non-irradiated cells lamin B is present in the nucleus as a 68-kDa protein. Irradiation causes its cleavage producing a 48-kDa fragment. The data obtained from Western blot analysis indicated that apoptosis was induced within 6 and 16 h, depending on the dose

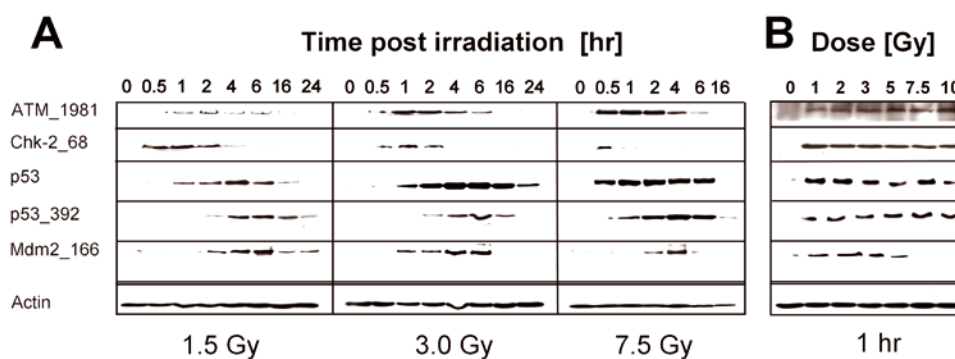


**Figure 1. Induction of apoptosis after gamma-irradiation.** MOLT-4 cells were irradiated by the doses of 1.5, 3.0, and 7.5 Gy and apoptosis was determined at the indicated times. A. Flow cytometric detection of apoptotic cells. Annexin V-positive cells were determined by APOPTEST (DakoCytomation), early apoptotic cells were measured as sub-G1 cells of DNA content analysis and late apoptotic cells were determined as APO2.7-positive ones. Each point represents the average of three independent experiments  $\pm$  S.E.M. B. Western blots analysis of lamin B. A 68-kDa nuclear protein is cleaved into a 48-kDa fragment when apoptosis is induced. Representative blots are shown.

of IR, since cleaved lamin B was detected 16 h after 1.5 Gy, and a small amount of the cleaved fragment could also be detected after 6 h after the doses of 3.0 or 7.5 Gy, respectively (Fig. 1B). Interestingly, after irradiation by the lethal dose of 7.5 Gy we did not find cleaved lamin B earlier than we did after the sublethal dose of 3.0 Gy, that is after 6 h, although this cleavage was more intense.

### Phosphorylation of ATM and Chk-2

In our experiments on MOLT-4 cells we found an increased amount of phosphorylated ATM (Ser-1981) as soon as 0.5 h after irradiation. This phosphorylation declined after 6 h after irradiation by the doses of 1.5 Gy, 3.0 or 7.5 Gy (Fig. 2A), i.e. just before the time when lamin B was cleaved and apoptosis was induced. After 0.5 h there was less active ATM after 1.5 or 3.0 Gy than after 7.5 Gy. Chk-2 was also found to be phosphorylated (Thr-68) within 0.5 h, but, unlike for ATM, its amount at that time was not affected by the dose. While the persistence of the phosphorylated form of ATM was the same (up to 6 h) after all the doses tested, phos-



**Figure 2. Gamma-radiation induced phosphorylation of ATM and its downstream targets.**

Representative Western blots are shown. A. MOLT-4 cells were irradiated by the doses of 1.5, 3.0, and 7.5 Gy and analysed at the times as indicated. IR induced rapid phosphorylation of ATM and Chk-2 with subsequent induction and phosphorylation of p53 and concomitant phosphorylation of Mdm2. These processes were followed by induction of apoptosis confirmed by detection of cleavage of lamin B (see Fig. 1B). B. MOLT-4 cells were irradiated by the doses as indicated and analysed 1 h after irradiation. Neither ATM nor its substrates exhibited a dose-dependent response. Only the amount of phosphorylated Mdm2 at Ser-166 was increased after the exposure to the doses up to 3 Gy, but after the dose of 5 Gy or higher it was decreased.

phorylation of Chk-2 (Thr-68) was detected up to 4, 2, and 1 h after 1.5, 3.0, and 7.5 Gy, respectively, and it preceded the accumulation of p53 (Fig. 2A). Although the amount of ATM after 0.5 h appeared to increase with an increasing dose, we did not observe any significant dose-dependence after 1 h after the exposure to the doses of 1–10 Gy, and also phosphorylation of Chk-2 did not exhibit a dose-dependent response 1 h post-irradiation (Fig. 2B).

### Phosphorylation of p53

The p53 protein was up-regulated from 1 up to 16 h after the dose of 1.5 Gy with a maximum at 4 h. After the dose of 3.0 Gy the up-regulation of p53 was detectable from 1 to 24 h and it was more intense, with a maximum also at 4 h. The dose of 7.5 Gy induced p53 very rapidly and transiently (from 0.5 to 6 h) with a maximum within 1–2 h after irradiation (Fig. 2A). p53 was subsequently phosphorylated on Ser-392 beginning 2 h after the dose of 1.5 and 3.0 Gy, or 0.5 h after 7.5 Gy. The phosphorylation on Ser-392 persisted up to 16 h after irradiation by 1.5 and 3.0 Gy. After 7.5 Gy it declined after 6 h.

At the time of maximal phosphorylation of p53 we observed an increase in phosphorylated oncoprotein Mdm2 (Ser-166), i.e. from 4 to 6 h after the dose of 1.5 or 3.0 Gy, or from 2 to 4 h after 7.5 Gy (Fig. 2A).

Similarly to ATM and Chk-2, neither induction of p53 nor its phosphorylation on Ser-392 exhibited a dose-dependent response 1 h after the exposure to the doses of 1 to 10 Gy (Fig. 2B). All proteins analysed were up-regulated after 1 h after gamma irradiation, nevertheless none of them but Mdm2

exhibited a dose-dependent response. One hour after the exposure to doses up to 3 Gy the amount of Mdm2 phosphorylated on Ser-166 increased, however, after the dose of 5 Gy or higher we observed a decrease in Mdm2 phosphorylation (Fig. 2B), suggesting that not even this protein might be exploited as a biodosimetric indicator.

### DISCUSSION

MOLT-4 cells are derived from human T-cell leukemia, with immunophenotypic characteristics of thymocytes, expressing CD1<sup>+</sup> (49%), CD4<sup>+</sup> (55%), CD5<sup>+</sup> (72%), and CD7<sup>+</sup> (77%) (Greenberg *et al.*, 1988) and they are a suitable model system to study molecular mechanisms of gamma-radiation-induced apoptosis of T-lymphocytes. Work on p53-null mice has revealed that p53 protein is important for induction of apoptosis as a response of T-lymphocytes to DNA damage (Clarke *et al.*, 1993). Our results together with the work of other authors (Nakano & Shinohara, 1999; Nakano *et al.*, 2001) prove that in MOLT-4 cells accumulation of p53 precedes typical apoptotic changes, such as fragmentation of DNA (sub-G1 peak), changes in plasma membrane phospholipids (annexin V), loss of plasma membrane integrity and mitochondrial changes (APO2.7), and cleavage of nuclear structural proteins (lamin B).

Induction of apoptosis in MOLT-4 cells (p53 wt) after exposure to doses up to 10 Gy is faster than in HL-60 cells (promyelocytic leukemia, p53-null). MOLT-4 cells show a wide disparity in the time when apoptosis is induced after gamma irradiation. The cells die mostly by delayed apoptosis and by post-mitotic apoptosis. The results of our previ-



ous work show that the sub-G1 peak is not induced before 6 h after irradiation by doses up to 5 Gy. The cells accumulate mainly in S phase and less in G2 phase (not shown).

Figure 1A compares the percentage of apoptosis determined by three methods 24 h after irradiation — annexin V-positive, APO2.7-positive (without permeabilization) and sub-G1 cells. A comparison of these methods indicates that determination of annexin V-positive and APO2.7-positive cells shows approximately the same results (thus, 24 h after irradiation the plasma membrane of the majority of apoptotic cells was permeable). The third method, determination of sub-G1 cells, provides much different data. After the doses of 3.0 and 7.5 Gy we determined roughly the same fraction of early apoptotic cells, 15.1% and 18.4%, respectively. It seems that MOLT-4 cells quickly form apoptotic bodies, decompose and the flow-cytometer evaluates the resulting fragments as debris.

After 24 h almost all the cells irradiated by the dose of 7.5 Gy were APO2.7-positive (without permeabilization), their plasma membrane was permeable at that time and they were apoptotic. Endlich *et al.* (2000) reported that only 24% of MOLT-4 cells underwent apoptosis without attempting cell division after a dose of 4 Gy. Most of the cells did not die until 18 to 30 h, however, mitosis either failed or the division was aberrant. We noticed the beginning of apoptosis (according to cleavage of nuclear lamin B) 16 h after 1.5 Gy and after 6 h after 3.0 or 7.5 Gy, respectively. Endlich *et al.* (2000) observed that the apoptotic death process ended by formation of apoptotic bodies 36 to 60 h post-irradiation.

To date, the proteins that directly sense DNA damage in mammalian cells are largely unidentified and the mechanism by which these sensors transmit DNA damage recognition to ATM remains to be elucidated. Bakkenist and Kastan (2003) in their work on fibroblasts suggested that introduction of DSB causes a rapid change of a higher-order chromatin structure, and that this chromatin alteration initiates ATM activation. ATM responds primarily to DSB, whilst other forms of DNA damage, such as UV radiation-induced photoproducts and replication fork stalling signal through the ATM- and Rad3-related (ATR) kinases. Recent studies have demonstrated that ATM functions upstream of ATR following exposure to IR in S/G2 (Stiff *et al.*, 2006) and they both exhibit selective substrate specificity in response to different genotoxic agents (Helt *et al.*, 2005). In addition, Bekker-Jensen *et al.* (2006) have reported that ATM is spatially redistributed to DSB-flanking chromatin and ATR accumulates by single-stranded DNA. Two other kinases, Chk-1 and Chk-2, do not concentrate around the DNA damage sites but rapidly spread to the entire nucleus. Chk-1 and Chk-2

are structurally unrelated yet functionally overlapping serine/threonine kinases. Chk-1 is a labile protein, active even in unperturbed cell cycles, since it plays an important role in keeping Cdc25C prepared for responding to DNA damage by phosphorylating its Ser-216 during the S to M phase (Kaneko *et al.*, 1999; Zhao *et al.*, 2002). In contrast, Lukas *et al.* (2001) showed that Chk-2 is a relatively stable protein responding to gamma-radiation throughout the cell cycle and it appears to be inactive in the absence of DNA damage. Nevertheless, recent findings of specific target knockdowns (Xiao *et al.*, 2006) suggest that Chk-1 is the only checkpoint kinase relevant as a cancer drug target. Our study shows that MOLT-4 cells, having wt p53, exhibit the same response to gamma-irradiation as fibroblasts do, i.e. they phosphorylate ATM and Chk-2 quickly after irradiation.

We found ATM to be phosphorylated early after irradiation and this phosphorylation persisted 6 h. Later (after irradiation by all the doses tested) the amount of ATM decreased. This was due to the fact that ATM is degraded before DNA fragmentation in the later stages of apoptosis resulting in the predominance of DNA-dependent protein kinase (DNA-PK). This is consistent with the results of Mukherjee *et al.* (2006), who proved that H2A.X (one of the core histones phosphorylated by ATM within the first minutes after irradiation) is phosphorylated in the later stages of apoptosis solely by DNA-PK, while ATM is dispensable for this process. Matsuoka *et al.* (2000) showed on fibroblasts that phosphorylated ATM subsequently phosphorylates Chk-2 on Thr-68. Here we show that after 0.5 h the amount of active ATM after 1.5 or 3.0 Gy was significantly less than after 7.5 Gy, nevertheless, even that amount was sufficient to activate Chk-2, which was phosphorylated within 0.5 h too.

Exposure to IR causes damage to DNA in the form of DSBs. This immediately activates ATM, which is involved in the cell cycle regulation and DNA repair and further conducts the signal up to p53 protein. It is well known that p53 possesses protective functions, such as stimulation of nucleotide excision repair and induction of cell cycle arrest (*via* other gene products) that is needed in order to check and repair the DNA damage. In contrast to these protective properties, p53 has also been implicated in the induction of apoptosis in certain cell types following DNA damage (reviewed in Ljungman, 2000). Work of Nakano *et al.* (2001) and our previous work (Szkanderova *et al.*, 2003) showed the radiation-induced apoptosis in MOLT-4 cells to be fully p53-dependent.

Both ATM and Chk-2 contribute to the precise control of an important element of the cellular emergency team, the p53 protein. Acting as a transcription factor for a number of downstream genes

that mediate either cell cycle arrest or apoptosis, p53 plays a critical role in maintaining genomic integrity after cellular stress by preventing propagation of damaged DNA. Nakano *et al.* (1999) and Szkannderova *et al.* (2003) suggested that the level of p53 must reach a threshold for the cells to undergo apoptosis.

Protein p53 was up-regulated after 1 h after irradiation by the cytostatic or the sublethal dose (1.5 and 3.0 Gy). The lethal dose (7.5 Gy) induced up-regulation of p53 very quickly (within 0.5 h, which was the time of activation of ATM and Chk-2). This rapidity reflects the emergency state in the cell after a large DNA insult and the need to urgently induce enough p53 to stop the cell cycle and to try to repair the damage or induce apoptosis.

But the up-regulated p53 does not function properly until post-translationally modified. The carboxyl terminus of p53 functions as an allosteric regulator. Phosphorylations on Ser-315 and Ser-392 localized within this domain enhance its sequence-specific DNA binding (Criswell *et al.*, 2003). Casein kinase 2 (CK2) phosphorylates p53 on Ser-392, but Claudio *et al.* (2006) demonstrated that Cdk9 itself is capable of such phosphorylation independently of CK2. Sakaguchi *et al.* (1997) showed that phosphorylation of Ser-392 stabilizes the tetramer formation of p53 that is critical for p53's ability to activate transcription because it facilitates phosphorylation of the transactivation domain due to a more favourable conformation. In this study, we also demonstrated that p53 was phosphorylated on Ser-392 and this phosphorylated form persisted 16 h after the cytostatic or the sublethal dose and 6 h after the lethal one. We showed in our previous work (Szkannderova *et al.*, 2003) that IR causes in MOLT-4 cells also phosphorylation on Ser-15 and both phosphorylations on Ser-392 and on Ser-15 show similar temporal patterns (they both decline after 6 h).

Whilst Bekker-Jensen *et al.* (2006) showed that U2OS human osteosarcoma cells treated with a DSB-generating insult (1–10 Gy) respond after 1 h by a homogeneous increase of phosphorylated p53 (Ser-15) throughout the entire nucleus, our experiments revealed no dose-dependent response of phosphorylation of p53 (Ser-392) 1 h after the exposure to the doses of 1 to 10 Gy (Fig. 2B).

Our results indicate that molecules involved in downstream pathways activated by ATM rather than ATM itself and its direct downstream targets would be suitable for biodosimetry in practice. ATM and its substrates might be used only in the low dose-range, although for reliable biodosimetry not a single one, but several biological indicators of the absorbed dose should be exploited.

We proved that MOLT-4 cells (p53 wt) respond to the IR-induced damage by phosphorylation

of ATM and Chk-2 (within 0.5 h after irradiation). We also proved that phosphorylation of p53 protein (2 h after irradiation) is accompanied by phosphorylation of its negative regulator, Mdm2 protein, which occurs concomitantly with maximal p53 phosphorylation.

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