*Proc. Natl. Acad. Sci. USA*
Vol. 89, pp. 9005–9009, October 1992
Medical Sciences

# Ionizing radiation stimulates unidentified tyrosine-specific protein kinases in human B-lymphocyte precursors, triggering apoptosis and clonogenic cell death

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*Communicated by E. Donnall Thomas, June 1, 1992*

**ABSTRACT** **Very little is known regarding the effects of ionizing radiation on cytoplasmic signal transduction pathways. Here, we show that ionizing radiation induces enhanced tyrosine phosphorylation of multiple substrates in human B-lymphocyte precursors. This response to ionizing radiation was also observed in cells pretreated with vanadate, a potent protein-tyrosine-phosphatase (PTPase) inhibitor, and phosphotyrosyl [Val$^5$]angiotensin II phosphatase assays showed no decreased PTPase activity in irradiated cells. Thus, enhanced tyrosine phosphorylation in irradiated B-lymphocyte precursors is not triggered by inhibition of total cellular PTPase activity. Immune-complex kinase assays using anti-phosphotyrosine antibodies demonstrated enhanced protein-tyrosine kinase (PTK) activity in the immunoprecipitates from irradiated cells, and the PTK inhibitors genistein and herbimycin effectively prevented radiation-induced tyrosine phosphorylation. Immune-complex kinase assays on irradiated and unirradiated B-lymphocyte precursors using antibodies prepared against unique amino acid sequences of p59$^{fyn}$, p56/p53$^{lyn}$, p55$^{blk}$, and p56$^{lck}$ demonstrated that these Src-family tyrosine kinases were not the primary PTKs responsible for enhanced tyrosine kinase activity in the anti-phosphotyrosine antibody immunoprecipitates or for enhanced tyrosine phosphorylation of multiple substrates. Thus, our findings favor the hypothesis that ionizing radiation induces enhanced tyrosine phosphorylation in B-lymphocyte precursors by stimulation of as yet unidentified PTKs. Tyrosine phosphorylation appears to be an important proximal step in radiation-induced apoptosis and clonogenic cell death because inhibition of PTK prevents DNA fragmentation and loss of clonogenicity of irradiated B-lymphocyte precursors. Since PTKs play myriad roles in the regulation of cell function and proliferation, the activation of a PTK cascade, as detailed in this report, may explain some of the pleiotropic effects of ionizing radiation on cellular functions of B-lymphocytes and their precursors.**

The molecular mechanism by which ionizing radiation inhibits and destroys mammalian cells has been widely explored but not precisely deciphered (1, 2). Protein-tyrosine kinases (PTKs) participate and likely play pivotal roles in initiation of signal cascades that affect proliferation and survival of human B-lymphocyte precursors (3–7). The purpose of this study was to examine the effects of ionizing radiation on PTKs in human B-lymphocyte precursors at discrete developmental stages of B-cell ontogeny.

## MATERIALS AND METHODS

**Patient Material and Cell Lines.** We used the fetal liver pro-B cell line FL112, the pre-pre-B cell line Reh, the pre-B cell line Nalm-6, and the early B/Burkitt lymphoma cell lines Daudi, Ramos, and Ramos-1 (a subclone of Ramos). The immunophenotypic and genotypic features and the radiation sensitivity of these human B-lymphocyte precursor cell lines were detailed in a previous report (8). We also used primary bone marrow blasts from a pre-pre-B acute lymphoblastic leukemia patient (UPN10), following the guidelines of the University of Minnesota Committee on the Use of Human Subjects in Research for secondary use of pathologic or surgical tissue.

**Irradiation of Cells and the Use of Kinase Inhibitors.** Cells ($5 \times 10^5$ per ml in plastic tissue culture flasks) were irradiated ($^{137}$Cs irradiator; J. L. Shephard, Glendale, CA, model Mark I) with 100–400 cGy at a dose rate of 99 cGy/min during logarithmic phase and under aerobic conditions, as previously described (8, 9). Prior to irradiation, cells were incubated for 1 hr at 37°C with (*a*) phosphate-buffered saline, (*b*) the PTK inhibitor genistein (10–100 μg/ml = 37–370 μM; ICN), (*c*) the protein kinase C inhibitor 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7; 11 μg/ml = 30 μM; GIBCO BRL), or (*d*) the protein-tyrosine-phosphatase (PTPase) inhibitor sodium orthovanadate (10–100 μM, Sigma) or for 24 hr at 37°C with the benzoquinoid ansamycin antibiotic herbimycin A (7 μg/ml = 12 μM; GIBCO/BRL), a potent PTK inhibitor, using previously described protocols (6, 7).

**Immunoblot Analysis of PTK Activation.** Human lymphocyte precursor cells ($5 \times 10^6$ per sample) were irradiated and then lysed 2 min after irradiation (6, 7). Activation of PTKs in these cells was measured by immunoblotting with a highly specific polyclonal rabbit anti-phosphotyrosine antibody to tyrosine-phosphorylated proteins in whole cell lysates, as described (6, 7).

**Measurement of Total Cellular PTPase Activity.** Two minutes after irradiation, phosphotyrosyl [Val$^5$]angiotensin II phosphatase assays were performed by the method of Minami *et al.* (10) with minor modifications. $^{32}$P-labeled [Val$^5$]angiotensin II peptides were prepared as described (10), except that recombinant p43$^{abl}$ (Oncogene Science, Manhasset, NY) was used instead of the p56$^{lck}$ activity of LSTRA cells to phosphorylate the [Val$^5$]angiotensin II peptide with [γ-$^{32}$P]ATP (4000–5000 Ci/mmol; 1 Ci = 37 GBq).

**Immune-Complex Kinase Assays.** Cells were irradiated with 100–400 cGy of γ-rays at 99 cGy/min and lysed 2 min later on ice with NP-40 lysis buffer [50 mM Tris, pH 8/150 mM NaCl/5 mM EDTA/1% (vol/vol) Nonidet P-40/100 μM

Abbreviations: PTK, protein-tyrosine kinase; PTPase, protein-tyrosine-phosphatase; kU, kilounits.

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sodium orthovanadate/100 μM sodium molybdate/500 μM phenylmethanesulfonyl fluoride containing aprotinin (8 μg/ml) and leupeptin (5 μg/ml)] and centrifuged for 10 min at 13,000 × *g* to remove insoluble material. Samples were immunoprecipitated with antisera prepared against unique amino acid sequences of the Src-family tyrosine kinases $p59^{fyn}$, $p56/p53^{lyn}$, $p55^{blk}$, and $p56^{lck}$ (11), which were kindly provided by Joseph Bolen (Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ). The antisera were used at a dilution of 1:500 and immune complexes were collected by incubation with 15 μl of protein A-Sepharose. After four washes with NP-40 lysis buffer, the protein A-Sepharose beads were washed once in kinase buffer (20 mM Mops, pH 7/10 mM $MgCl_2$) and resuspended in the same buffer. Reactions were initiated by the addition of 3 μg of acid-denatured rabbit muscle enolase (12) (Sigma), 25 μCi of [γ-$^{32}$P]ATP (5000 Ci/mmol), and unlabeled ATP to a final concentration of 5 μM. Reactions were terminated by boiling for 4 min in SDS sample buffer. Samples were then electrophoresed in SDS/9.5% polyacrylamide gels and labeled proteins were detected by autoradiography. Immune-complex kinase assays were performed as described above, except that affinity-purified rabbit anti-phosphotyrosine antibodies (5 μg/ml) were used for immunoprecipitation.

**Apoptosis Assays.** Cells were harvested 4 hr after exposure to ionizing radiation in the presence or absence of the PTK inhibitor genistein (1-hr preincubation and continued presence during irradiation at a concentration of 10 μg/ml), and DNA was prepared for analysis of fragmentation. DNA was then electrophoresed through a 1% agarose gel and the DNA bands were visualized by UV light after staining with ethidium bromide (0.5 μg/ml). *Hae* III-digested φX174 replicative-form DNA mixed 1:1 with *Hin*dIII-digested λ DNA (DNA concentration, 400 μg/ml in 10 mM Tris·HCl, pH 7.4/5 mM NaCl/0.1 mM EDTA) was used for sizing the DNA fragments on the ethidium bromide-stained agarose gels.

**Clonogenic Assays.** To evaluate the role of tyrosine phosphorylation in clonogenic cell death after radiation exposure, cells were irradiated with 100–200 cGy of γ-rays at 99 cGy/min in the presence or absence of the PTK inhibitor genistein (1-hr preincubation at 37°C and continued presence during radiation exposure at a concentration of 10 μg/ml) or in the presence of the PTPase inhibitor sodium orthovanadate (1-hr preincubation at 37°C and continued presence during radiation exposure at a concentration of 10 μM) and analyzed for clonogenic growth by *in vitro* methylcellulose colony assays (6, 8, 13, 14).

## RESULTS AND DISCUSSION

**Ionizing Radiation Induces Tyrosine Phosphorylation in Human B-Lymphocyte Precursors by Stimulation of Unidentified PTKs.** Exposure of Nalm-6 (pre-B), Ramos (early B), Daudi (early B), and Ramos-1 (early B) cells to ionizing radiation increased tyrosine phosphorylation of multiple electrophoretically distinct phosphoproteins (Fig. 1). Phosphoproteins with apparent molecular masses of 34, 55, 69, 76, 97, and 150 kDa were strongly phosphorylated on tyrosine and low levels of tyrosine phosphorylation were induced on substrates of 40, 46, 50, 53, 59, 62, 120, and 190 kDa. The radiation-triggered induction of tyrosine phosphorylation was effectively prevented by the PTK inhibitors herbimycin and genistein but not by the protein kinase C PKC inhibitor H7. Tyrosine phosphorylation was markedly enhanced by the PTPase inhibitor vanadate. Similar results were obtained with FL112 (fetal liver pro-B) or Reh (pre-pre-B) cells (data not shown).

The enhanced tyrosine phosphorylation observed in irradiated lymphocyte precursors could be due to PTK activation, PTPase inactivation, or a combination of these two mechanisms. Therefore, we measured the total cellular PTPase activity in irradiated cells using [$^{32}$P]phosphotyrosyl [Val$^5$]angiotensin II as a phosphatase substrate (10). Treatment of Reh (pre-pre-B) or Ramos (early B) cells with the PTPase inhibitor sodium orthovanadate alone or with sodium orthovanadate plus hydrogen peroxide abolished the whole-cell PTPase activity, as measured by the specific release of $^{32}P_i$ from the $^{32}$P-labeled [Val$^5$]angiotensin II peptides (Table 1). In contrast, exposure to 100–400 cGy of γ-rays at 99 cGy/min did not substantially reduce PTPase activity in our

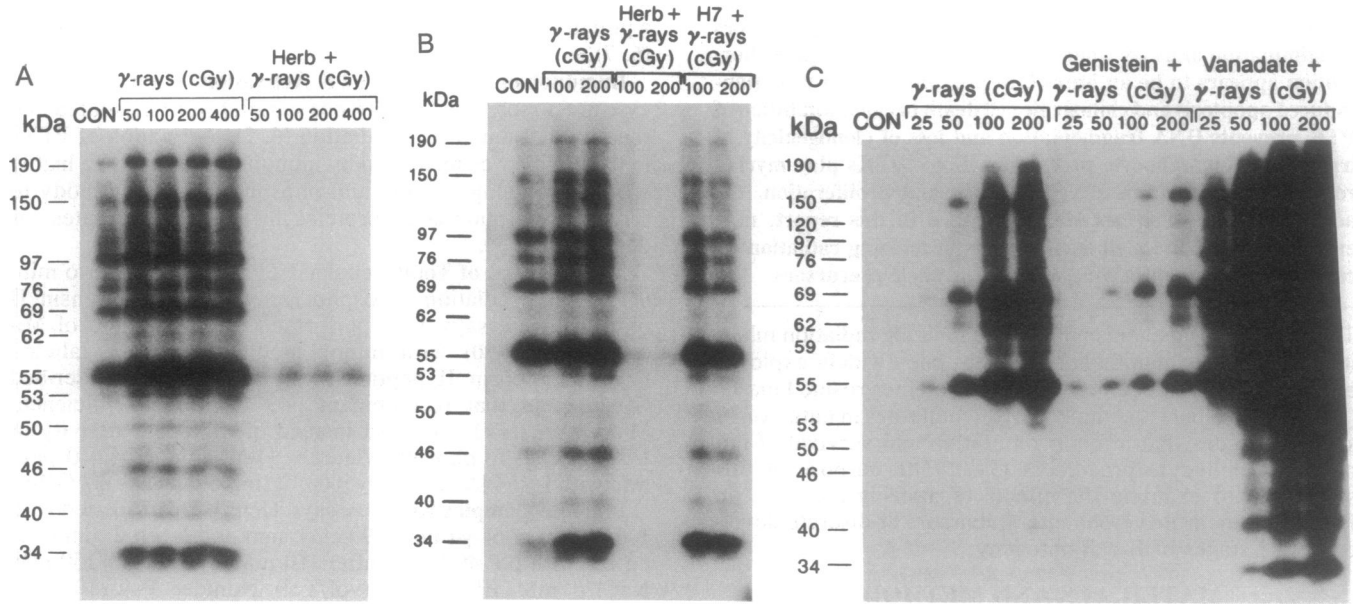


FIG. 1. Ionizing radiation induces tyrosine phosphorylation of multiple electrophoretically distinct cellular substrates in human B-lymphocyte precursors. Nalm-6 (pre-B) (*A*), Ramos (early B) (*B*), and Daudi (early B) (*C*) cells were irradiated in the presence or absence of the PTK inhibitors herbimycin (7 μg/ml = 12 μM) and genistein (100 μg/ml = 370 μM), the protein kinase C inhibitor H7 (11 μg/ml = 30 μM), and the PTPase sodium orthovanadate (100 μM). Two minutes after irradiation, cells were lysed with SDS sample buffer, and equivalent amounts of protein were loaded on a 10.5% polyacrylamide gel, electrophoresed overnight, transferred to Immobilon (Millipore), and incubated with anti-phosphotyrosine antibody (0.5 μg/ml) and $^{125}$I-labeled protein A before exposure to x-ray film, as described (6, 7). CON, control.

Table 1. Effects of ionizing radiation on PTPase activity in human B-lymphocyte precursors

| Cells and treatment | PTPase activity, cpm/mg of protein | % inhibition |
|---|---|---|
| Reh (pre-pre-B) | | |
| Control | 23,207 ± 2,294 | — |
| γ-Rays | | |
| 100 cGy | 23,780 ± 1,092 | 0.0 |
| 200 cGy | 18,186 ± 1,786 | 21.6 |
| Vanadate | 0 ± 0 | >99.99 |
| $H_2O_2$ + vanadate | 0 ± 0 | >99.99 |
| Ramos (early B) | | |
| Control | 151,000 ± 24,000 | — |
| γ-Rays | | |
| 100 cGy | 146,000 ± 4,000 | 3.3 |
| 200 cGy | 127,700 ± 5,000 | 15.4 |
| 400 cGy | 148,300 ± 31,900 | 1.8 |
| Vanadate | 0 ± 0 | >99.9 |
| $H_2O_2$ + vanadate | 0 ± 0 | >99.9 |

Whole-cell PTPase activity in irradiated B-lymphocyte precursor cell lines was determined in two independent experiments using phosphotyrosyl [Val$^5$]angiotensin II phosphatase assays, which measure the specific release of $^{32}$P from radiolabeled PTPase substrate [Val$^5$]angiotensin II. Vanadate was used at 100 μM, and hydrogen peroxide at 6 mM. Data are expressed as the total PTPase specific activity after the indicated treatments.

study (Table 1), indicating that the enhancement of tyrosine phosphorylation by ionizing radiation was not caused by a reduction of total cellular PTPase activity.

These observations prompted the hypothesis that ionizing radiation stimulates the activity of PTKs in human B-lymphocyte precursors. Therefore, we next evaluated in immune-complex kinase assays the effects of ionizing radiation on the enzymatic activity of the three members of the Src-related PTK family that are abundantly expressed in the cytoplasm of B-lineage cells: the *fyn*, *lyn*, and *blk* gene products (11). To this end, lysates of unirradiated or irradiated Ramos (early B) cells were immunoprecipitated with antisera against unique amino acid sequences of p59$^{fyn}$, p56/p53$^{lyn}$, and p55$^{blk}$. The PTK activity in the immunoprecipitates was measured by phosphorylation of acid-denatured rabbit muscle enolase, which was used as an exogenous substrate, as well as by autophosphorylation of the specific PTK in Ramos cells (Fig. 2). No stimulation of PTK activity was observed when immunoprecipitations were initially performed on lysates prepared 2 min after radiation exposure (data not shown). Therefore, cells were lysed within 30 sec after radiation exposure in all subsequent experiments in order to detect any early changes in activity. γ-Irradiation with 100–400 cGy induced consistent albeit very low-level activation of p59$^{fyn}$ [PhosphorImager analysis of phosphoenolase bands: 816 kilounits (kU) for 0 cGy, 952 kU for 100 cGy, 1157 kU for 200 cGy, and 1027 kU for 400 cGy] and p55$^{blk}$ (102 kU 0 cGy, 155 kU for 100 cGy, 152 kU for 200 cGy, and 150 kU for 400 cGy), whereas p56/p53$^{lyn}$ did not show any reproducible change in activity (512 kU for 0 cGy, 352 kU for 100 cGy, 525 kU for 200 cGy, and 472 kU for 400 cGy) (Fig. 2). Similar results were obtained with Ramos-1, and Daudi (early B) cell lines (data not shown). In another experiment, we examined the effects of ionizing radiation on p56$^{lck}$ activity in Ramos cells. γ-Irradiation did not stimulate p56$^{lck}$ activity (PhosphorImager analysis: p56$^{lck}$ autophosphorylation band, 530, 607, and 387 kU at 0, 100, and 200 cGy; phosphoenolase band, 2030, 2120, and 1875 kU at 0, 100, and 200 cGy) (Fig. 2).

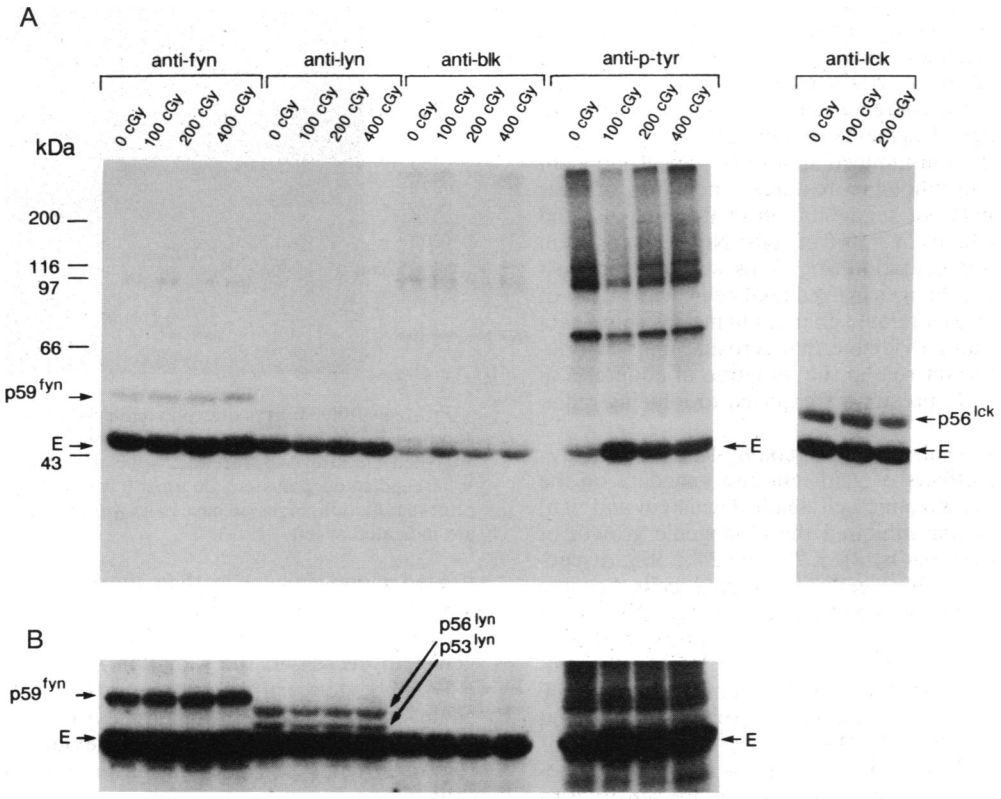


FIG. 2. Effects of ionizing radiation on enzymatic activity of Src-family PTKs and the anti-phosphotyrosine-reactive PTKs in human early B-cells. Immune-complex kinase assays were performed on Ramos cells, and proteins labeled by $^{32}$P were detected by autoradiography following SDS/polyacrylamide gel electrophoresis. Phosphorylation of the PTK substrate enolase (E) is shown from a 4-hr exposure of the gels (*A*), and autophosphorylation of the PTKs (positions indicated by arrows) is shown from a 16-hr exposure of the same gels (*B*). Positions of molecular size markers are shown at left. PTK activity was quantitated by PhosphorImager (Molecular Dynamics, Sunnyvale, CA). p-tyr, Phosphotyrosine.

PTK activation in irradiated Ramos cells was also examined by anti-phosphotyrosine immune-complex kinase assays using rabbit muscle enolase as exogenous substrate (Fig. 2). Ramos cells irradiated with 100 cGy showed a 4.8-fold increase in kinase activity (75% phosphotyrosine by phospho amino acid analysis of the enolase band) compared with unirradiated Ramos cells (PhosphorImager analysis of phosphoenolase bands: 1008 kU for 100 cGy vs. 208 kU for 0 cGy), whereas Ramos cells irradiated with 200 or 400 cGy showed >2-fold elevated PTK activity (576 or 491 kU). Thus, the level of PTK activity in anti-phosphotyrosine immunoprecipitates from irradiated Ramos cells did not correlate with radiation dose, perhaps because the cells exposed to higher radiation doses were analyzed later after initiation of radiation exposure than the 100-cGy samples (at the same dose rate, exposure to 200 or 400 cGy requires longer radiation exposure than 100 cGy), when the stimulated PTKs were no longer at their peak activity. Also, the level of PTK activity recovered was dependent on both the activation state of PTKs and their level of tyrosine phosphorylation.

Taken together, these findings provide direct evidence that ionizing radiation stimulates PTKs. The minimal and transient (not detectable when assayed 2 min after radiation exposure) increases in activity of $p59^{fyn}$ and $p55^{blk}$ after ionizing radiation suggest that these Src-family PTKs are not the primary kinases responsible for enhanced tyrosine phosphorylation of multiple substrates in irradiated cells. It is more likely that these PTKs become activated during a secondary signal-amplification step. The results of the anti-phosphotyrosine immune-complex kinase assays strongly favor the hypothesis that additional, as yet unidentified PTKs are activated in human B-lymphocyte precursors after treatment with ionizing radiation.

**Role of Tyrosine Phosphorylation in Radiation-Induced Apoptosis and Clonogenic Cell Death.** We next examined the role of tyrosine phosphorylation in radiation-induced apoptosis. DNA from unirradiated control cells showed no fragmentation whereas DNA from irradiated cells had a ladder-like fragmentation pattern characteristic of apoptosis, with oligonucleosome-length fragments at multiples of ≈200 base pairs (Fig. 3). Radiation-induced apoptosis was also evident from distinctive morphologic features, including nuclear chromatin condensation, segmentation of the nucleus, and plasma membrane blebs in >50% of cells. Notably, genistein prevented DNA fragmentation (Fig. 3) as well as apoptosis-related morphologic changes in irradiated cells, with <25% of cells showing apoptosis-related changes in morphology (data not shown), providing evidence that tyrosine phosphorylation plays an important role in the initiation of apoptosis in human B-lymphocyte precursors exposed to ionizing radiation.

To extend these results, we used colony-formation assays to investigate the effects of genistein and vanadate on the radiation-induced clonogenic cell death. Genistein and vanadate treatments alone inhibited the clonogenic growth of B-lymphocyte precursors by 25 ± 7% and 24 ± 9%, respectively (Table 2). Notably, genistein-treated cells became markedly resistant to radiation (Table 2). Whereas 200 cGy of γ-rays inhibited the clonogenic growth of untreated B-lymphocyte precursors by 71 ± 7%, only 28 ± 7% of genistein-treated cells were inhibited ($P = 0.002$). Similarly, while 100 cGy of γ-rays inhibited the clonogenic growth of untreated B-lymphocyte precursors by 43 ± 13%, only 5 ± 3% of genistein-treated cells were inhibited ($P = 0.02$). Vanadate-treated cells appeared to be significantly more sensitive to ionizing radiation than untreated cells: 100 cGy of γ-rays inhibited the clonogenic growth of B-lymphocyte precursors by only 43 ± 13%, but 93 ± 3% of vanadate-treated cells were inhibited after the same radiation dose ($P = 0.007$). Similarly, 200 cGy of γ-rays inhibited a greater fraction of vanadate-treated clonogenic cells than untreated cells (99 ± 0.2% inhibition for vanadate-treated cells versus 71 ± 7% inhibition for untreated cells, $P = 0.004$). Thus, the PTK inhibitor genistein decreased the radiation sensitivity of clonogenic B-lymphocyte precursors, whereas the PTPase inhibitor vanadate appeared to sensitize them to the lethal effects of ionizing radiation, providing further evidence for the cardinal role of tyrosine phosphorylation in the death of irradiated cells.

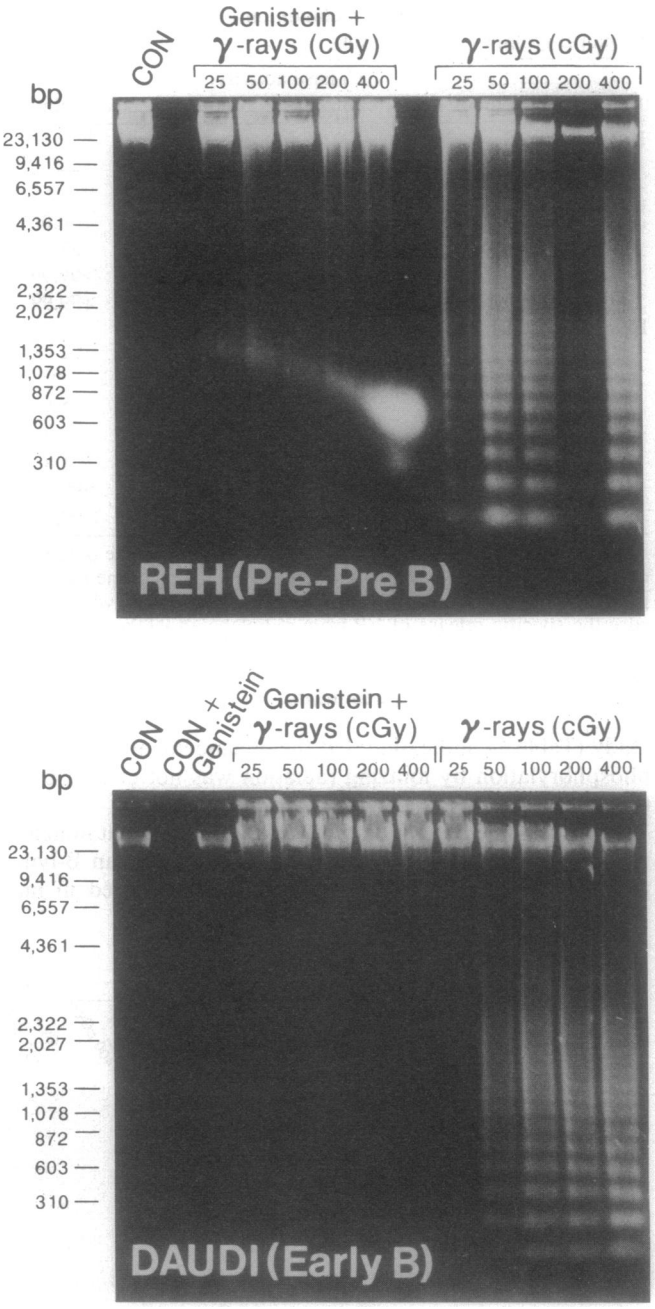


FIG. 3. Role of tyrosine phosphorylation in radiation-induced apoptosis. DNA fragmentation patterns of irradiated cells were analyzed 4 hr after exposure to 25–400 cGy of γ-rays at 99 cGy/min. Where indicated, genistein (30 μg/ml) was added to the cultures 1 hr prior to radiation. Sizes [in base pairs (bp)] of molecular size markers are indicated at left.

In conclusion, this study clarifies and expands our current knowledge of radiation-induced biochemical signals. Our evidence indicates a signaling cascade that is intimately linked to a PTK pathway leading to apoptosis and clonogenic cell death. Immune-complex kinase assays on irradiated and

Table 2. Role of tyrosine phosphorylation in radiation-induced death of clonogenic human lymphocyte precursor cells

| Treatment | Dose, cGy | Mean no. of colonies per $10^4$ cells (% death of clonogenic cells) FL112 (pro-B) | UPN10* (pre-pre-B) | Reh (pre-pre-B) | Ramos (early B) | Daudi (early B) | Mean % death of clonogenic cells |
|---|---|---|---|---|---|---|---|
| PBS, γ-rays | 0 | 125 (—) | 1074 (—) | 259 (—) | 794 (—) | 767 (—) | — |
| | 100 | 48 (62) | 987 (8) | 75 (71) | 681 (14) | 307 (60) | 43 ± 13 |
| | 200 | 21 (83) | 425 (60) | 24 (91) | 393 (51) | 240 (69) | 71 ± 7 |
| Genistein, γ-rays | 0 | 92 (—) | 759 (—) | 135 (—) | 684 (—) | 703 (—) | — |
| | 100 | 91 (1) | 760 (0) | 116 (14) | 592 (0) | 647 (8) | 5 ± 3 |
| | 200 | 75 (18) | 694 (9) | 79 (41) | 427 (27) | 396 (44) | 28 ± 7 |
| $VO_4$, γ-rays | 0 | 82 (—) | 1125 (—) | 223 (—) | 656 (—) | 353 (—) | — |
| | 100 | 1 (99) | 215 (81) | 24 (89) | 13 (98) | 12 (97) | 93 ± 3 |
| | 200 | 0 (>99) | 11 (99) | 0 (>99.6) | 1 (>99.8) | 0 (>99.7) | 99 ± 0.2 |

Cells were irradiated in phosphate-buffered saline (PBS) in the presence and absence of the PTK inhibitor genistein (30 μg/ml) or the PTPase inhibitors sodium orthovanadate (10 μM) as described (6, 7). Subsequently, cells ($10^4$ or $10^5$ per sample in duplicate samples) were assayed for colony formation (13, 14). Results are expressed as the mean number of colonies per $10^4/10^5$ cells plated and the % radiation-induced death of clonogenic cells. The % radiation-induced death was calculated as 100 − 100 (mean no. colonies in irradiated samples)/(mean no. of colonies in unirradiated samples). Genistein treatment alone without radiation inhibited clonogenic growth of FL112 cells by 26%, UPN10 cells by 29%, Reh cells by 48%, Ramos cells by 14%, and Daudi cells by 8% (mean ± SE, 25 ± 7%). Vanadate treatment alone without radiation inhibited clonogenic growth of FL112 cells by 34%, UPN10 cells by 0%, Reh cells by 14%, Ramos cells by 17%, and Daudi cells by 54% (mean ± SE, 24 ± 9%). Variation of colony numbers between the individual duplicate dishes did not exceed 10% of the mean values.
*$10^5$ cells.

unirradiated B-lymphocyte precursors using antibodies prepared against unique amino acid sequences of $p59^{fyn}$, $p56/p53^{lyn}$, $p55^{blk}$, and $p56^{lck}$ indicated that these Src-family tyrosine kinases were not the primary PTKs responsible for enhanced tyrosine kinase activity in the anti-phosphotyrosine immunoprecipitates or for enhanced tyrosine phosphorylation of multiple substrates. Thus, our findings favor the hypothesis that ionizing radiation induces enhanced tyrosine phosphorylation in B-lymphocyte precursors by stimulation of as yet unidentified PTKs. The identification and characterization of radiation-induced PTKs will require further investigation. Our observations raise the intriguing possibility that the activity of the endonuclease(s) responsible for radiation-induced apoptosis is directly regulated by tyrosine phosphorylation, which in turn is regulated by radiation-stimulated PTKs. Since PTKs play myriad roles in the regulation of cell function and proliferation (15, 16), the activation of a protein kinase cascade, as detailed in this report, may explain the pleiotropic effects of ionizing radiation on cellular functions. Furthermore, a delicate balance of oncogenic vs. tumor-suppressive proteins might be altered in irradiated cells when a tyrosine kinase regulatory pathway is activated (15, 16). These observations link radiation-triggered signaling events to the biologic effects of ionizing radiation. Our study provides insights for future identification of PTKs involved in the initiation of the radiation-induced transmembrane signal and their functionally important substrates.

This work was supported in part by Public Health Service Grants R29 CA42111, R01 CA42633, and R01 CA51425 from the National Cancer Institute, by special grants from the Minnesota Medical Foundation, Children's Cancer Research Fund, and Bone Marrow Transplant Research Fund, University of Minnesota, and in part by Bristol-Myers Squibb. F.M.U. is a Scholar of the Leukemia Society of America. This is publication no. 80 from the Tumor Immunology Laboratory, University of Minnesota.

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