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ARTICLES

Protein Kinase C ζ Nuclear Translocation Mediates the Occurrence of Radioresistance in Friend Erythroleukemia Cells

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Abstract Friend erythroleukemia cells require high doses (15 Gy) of ionizing radiation to display a reduced rate of proliferation and an increased number of dead cells. Since ionizing radiation can activate several signaling pathways at the plasma membrane which can lead to the nuclear translocation of a number of proteins, we looked at the intranuclear signaling system activated by Protein Kinases C, being this family of enzymes involved in the regulation of cell growth and death. Our results show an early and dose-dependent increased activity of ζ and ε isoforms, although PKC ζ is the only isoform significantly active and translocated into the nuclear compartment upon low (1.5 Gy) and high (15 Gy) radiation doses. These observations are concomitant and consistent with an increase in the anti-apoptotic protein Bcl-2 level upon both radiation doses. Our results point at the involvement of the PKC pathway in the survival response to ionizing radiation of this peculiar cell line, offering PKC ζ for consideration as a possible target of pharmacological treatments aimed at amplifying the effect of such a genotoxic agent. J. Cell. Biochem. 88: 144–151, 2003.

Key words: PKC ζ; radioresistance; Friend cells

Ionizing radiation (IR) can induce cell death by activating several signaling pathways at the plasma membrane among which stress-activated protein kinase (SAPK), c-jun-N-terminal kinase (JNK), ceramide reactive oxygen species and Protein Kinase C (PKC) [Olson et al., 1993; Buchner, 1995; Haimovitz-Friedman, 1998; Watters, 1999]. Actually PKC family includes 12 isoforms of serine/threonine kinases, ubiquitously expressed, involved in a wide range of physiological processes such as cell growth [Takeda et al., 1999], actin cytoskeleton organization [Keeman and Kelleher, 1998], apoptosis [Shimizu et al., 1998; Cross et al., 2000], differentiation [Mallia et al., 1999], neoplastic transformation [La Porta et al., 1997], and insulin signaling [Sajan et al., 1999]. Protein Kinases C are classified into three main groups characterized by phospholipids, Ca^{++} and DAG (diacylglycerol) requirement for activity. Conventional PKCs (α_1, β1, β2, γ) require PS, Ca^{++}, and DAG, novel PKCs (δ, ε, η, θ, μ) require only DAG and PS (phosphatidylserine), atypical PKCs (ζ, ι, λ) are activated by phospholipids such as PS or PI3, 4, 5P3 [Nakanishi et al., 1993; Newton, 1997; Mellor and Parker, 1998; Martelli et al., 1999; Cataldi et al., 2000]. Several of these isoforms, namely α, δ, ε, μ, and ζ, are involved in the initiation of stress-induced radioresistant or radiosensitive response in a variety of normal and neoplastic cells, depending on the type and state of the cell (normal or neoplastic) and on the dose administered [Kelly et al., 1998; Yuan et al., 1998; Lee et al., 1999; Niehoff and Uckun, 2000]. Thus, activation of PKCs can mediate in some circumstances the occurrence of cell death, while, in others, it protects the cell from apoptosis [Lavin et al., 1996]. Thus, we examined the expression and activation of PKC δ, ε, and ζ isozymes and their possible role in mediating the radioresistance displayed by Friend erythroleukemia cells [Einspanner et al., 1984] in order to

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set up against a specific molecular target pharmacological treatments aimed at amplifying the effect of IR.

MATERIALS AND METHODS

Cell Cultures and Irradiation Protocol

Friend erythroleukemia cells were grown in suspension in RPMI-1640, 10% FCS, glutamine, HEPES, penicillin/streptomycin in a humidified atmosphere plus 5% CO_2 and irradiated at room temperature by a Mevatron 74 Siemens linear accelerator (photonic energy: 10 MV) administering 1.5 and 15 Gy (dose rate 3 Gy/min). Cell viability was assessed by the Trypan blue dye exclusion test. For Western blotting analysis, immunoelectronmicroscopy and in vitro PKC activity, samples were processed for 1 h after IR exposure.

TUNEL Analysis

TUNEL analysis was performed at 4 h after IR exposure in order to detect early apoptosis. Cells were cytospinned, fixed in paraformaldehyde (4% v/v in PBS pH 7.4) for 30 min at room temperature and then incubated in a permeabilizing solution (0.1% Triton X-100, 0.1% sodium cytrate) for 2 min on ice. DNA strand breaks were identified by labeling the free 3′-OH nucleotide termini with fluorescein-dUTP with the In situ Cell Death Detection Kit (Boehringer Mannheim, Germany) as described by the manufacturer. Slides were counterstained with propidium iodide, mounted in glycerol and analyzed. The extent of DNA fragmentation was quantitated by direct visual counting of green fluorescent labeled nuclei at 40× magnification. Five slides were examined per sample and apoptotic cells were scored out of a total of 100 cells.

Electron Microscopy

Samples left for 18 h in post-irradiation culture were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.6, for 60 min at 4°C and post-fixed in 1% osmium tetroxide for 60 min at 4°C. Samples were then dehydrated in alcohol at progressively higher concentrations and embedded in Spurr resin (Electron Microscopy Sciences, Fort Washington, WA).

Immunoelectronmicroscopy

Cells were fixed in a mixture of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.6, for 60 min at 4°C. Samples were dehydrated in alcohol at progressively higher concentrations, embedded in hydrophilic Bioacryl resin (British Biocell Intl, Cardiff, United Kingdom), followed by UV polymerization. Ultrathin sections were cut by using a Reichert ultramicrotome and mounted on 300 mesh nickel grids. To block non-specific binding sites, grids were treated with a blocking buffer made of PBS supplemented with 0.1% Tween-20, 0.1% bovine serum albumin (BSA), 1% non-fat dried milk, 3% normal goat serum (NGS) incubated overnight in the presence of a rabbit PKC ζ polyclonal antibody, followed by a 30 min incubation with anti-rabbit IgG conjugated with 15 nm colloidal gold particles (British BioCell, Cardiff, United Kingdom). Grids were then counterstained in uranyl acetate and lead citrate to preserve cell morphology and photographed by using a Zeiss electron microscope 109.

Cell Fractionation

Cells were resuspended in 10 mM Tris-HCl pH 7.4, 10 mM NaCl, 2 mM $MgCl_2$, 0.6% Triton X-100, 1.0 mM PMSF, 1 μg/ml leupeptin and aprotinin, 1.0 mM Na_3VO_4, incubated at room temperature for 2 min and cooled on ice for 5 min. After four passages through a 22-gauge needle, the concentration of $MgCl_2$ was adjusted to 5 mM. Nuclei were obtained by centrifuging the suspension at 600*g* for 5 min and cytoplasmic fractions consisted of postnuclear supernatants. Nuclear purity was assessed by detection of beta actin and only nuclei showing a complete absence of beta actin in Western blots were used in the reported experiments.

Western Blotting Analysis

Proteins (10 μg) from total homogenates and nuclear or cytoplasmic fractions were SDS–PAGE separated and transferred to nitrocellulose. After blocking in 5% non-fat milk, 10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween-20, the membranes were probed with rabbit PKC δ, ε, ζ polyclonal or mouse Bcl-2 monoclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) and labeled with enzyme conjugated anti-rabbit IgG or anti-mouse horseradish peroxidase. Bands were detected with ECL detection system (Amersham Intl., United Kingdom).

In Vitro PKC Activity Assay

Total cellular proteins (500 μg) were supplemented with 2 μg of rabbit PKC ε or ζ polyclonal

antibody and 20 μl of Protein G-Agarose. Immunoprecipitated proteins were collected by centrifugation, washed twice in PBS, and incubated at 30°C for 10 min in 20 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 10 μM ATP, 5 μCi [γ^{32}P] ATP, 40 μg/ml PS, 3.3 μM dioleylglycerol, and 10 μg of the appropriate synthetic peptide QKRPSQRSKYL, corresponding to a sequence derived from myelin basic protein (MBP) (Santa Cruz lab., Santa Cruz, CA). Dioleylglycerol was omitted when assaying the DAG-independent PKC ζ. Proteins, resuspended in 2× sample buffer, were separated through 18% SDS–PAGE. Gels were then dried and exposed to X-ray films for 16 h at −80°C. Peptide spots were excized and radioactivity was measured in a liquid scintillation counter.

Image Processing and Analysis System

Quantitative analysis of the Protein kinases C expression and activity was performed by using a Sony videocamera connected to a Leica Quantimet 500 plus software (Leica Cambridge Ltd., Cambridge, United Kingdom) determining the change in Integrated Optical Intensity (IOI) using ISO transmission density Kodak CAT 152-3406 (Eastman Kodak Company, Rochester, NY) as standard. Results were expressed as mean ± SD. Statistical analysis was performed using the analysis of variance (ANOVA). Probability of null hypothesis of <0.1% ($P < 0.05$) was considered statistically significant.

RESULTS

Friend erythroleukemia cells were exposed to low (1.5 Gy) and high (15 Gy) doses, chosen on the basis of the standard radiotherapeutic schemes used for the management of various human cancers. In fact, a daily fraction of 1.5 Gy or more is commonly delivered in human tumor radiotherapy, whereas 15 Gy can be reached at the end of a therapeutic protocol or occasionally used as a single fraction for palliative treatments [Chinnayian et al., 2000]. Two independent methods were used to evaluate the cytotoxic effect of IR: Trypan blue dye exclusion test to detect viable cells and TUNEL analysis to score apoptotic cells. Friend cells require high radiation doses to show an inhibited cell growth and an evident reduction in viability, which peaks at day 6 after exposure (Fig. 1). An inhibited growth after the higher

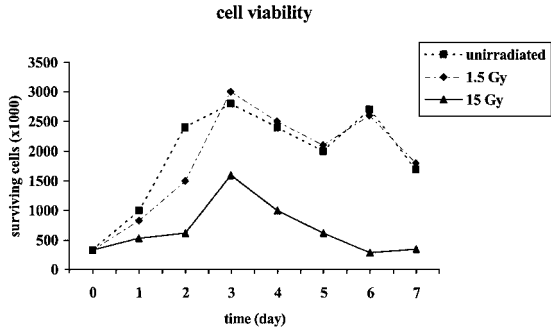

Fig. 1. Effect of IR on cell growth and viability of Friend cells. Results are the mean ± SD of three different experiments.

dose is detected also in human acute lymphoblastic cells (Jurkat), but these cells, differently from Friend, disclose a 90% reduction of Trypan blue excluding cell number at the 5th day which confirms their strong sensitivity to IR [Cataldi et al., 2002b]. These cytostatic/cytotoxic effects, found in Friend cells, are justified by the already reported accumulation in the S/G2-M phase of the cell cycle [Di Pietro et al., 2001] and by the dose dependent increase in cell necrosis and apoptosis (Fig. 2). The latter was quantified by TUNEL analysis at 4 h upon irradiation, since this is the earliest time to reveal DNA breaks in hemopoietic cells [Negoescu et al., 1996], while the morphological hallmarks of apoptosis were documented at 18 h by electron microscopy techniques. In spite of the high percentage of early apoptosis already detectable at 4 h upon irradiation (40% upon 15 Gy; 10% upon 1.5 Gy; 1% in unirradiated samples) and the clear drop in cell growth, maximal at day 6, cells from 15 Gy samples were able to survive and start growing again for more than 1 month in post-irradiation culture (not shown). This observation was consistent with a doubling in anti-apoptotic molecule Bcl-2 expression in total homogenates derived from cells exposed to 15 Gy IR compared to unirradiated cells (2.5 ± 0.30 vs. 1.26 ± 0.10) (Fig. 3). Since the activation of specific Protein Kinases C can in some circumstances protect cells from death, while in other cases it mediates the occurrence of apoptosis [Lavin et al., 1996], we next sought to investigate the involvement, if any, of PKC δ, ε, and ζ in the cellular response to IR. As shown in Figure 4, Western blotting analysis displayed a dose-dependent upregulation of PKC ζ in total homogenates obtained 1 h after irradiation. Densitometric analysis of Western blots revealed a 2.3-fold increased expression of the ζ isoform after 15 Gy, whereas

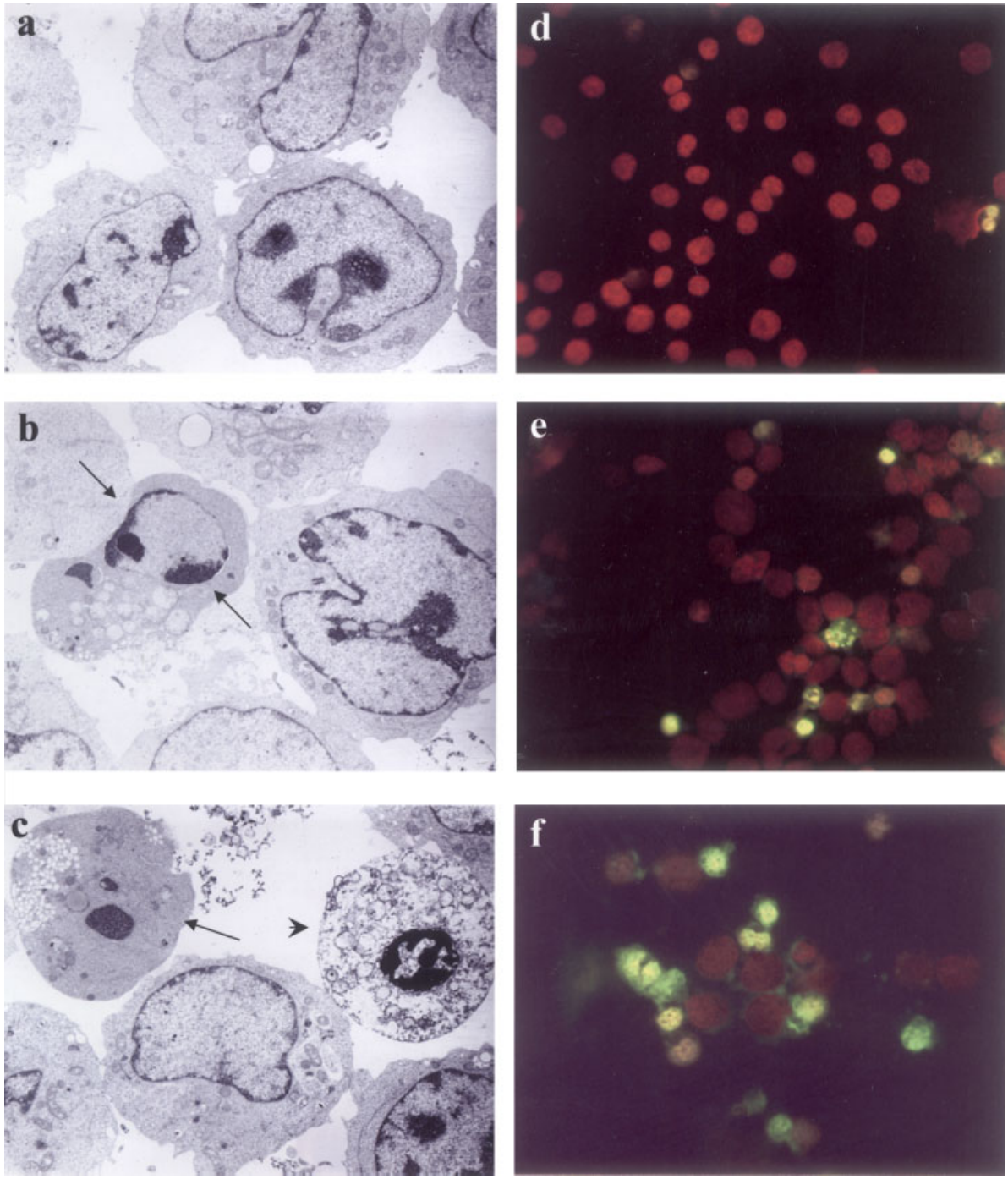

Fig. 2. Morphological analysis of Friend cells after exposure to 1.5 and 15 Gy IR. Transmission electron microscopy analysis was performed 18 h after IR exposure. **a**: Unirradiated, (**b**) 1.5 Gy (**c**) 15 Gy. Arrows indicate early (b) and late (c) apoptosis. Note the typical features of chromatin margination, micronuclei and cell shrinkage. Arrowhead points at a necrotic cell. Magnification: 3000×. TUNEL detection. **d**: Unirradiated, (**e**) 1.5 Gy, (**f**) 15 Gy. Green fluorescence (FITC) is related to apoptotic DNA and red fluorescence (PI) is related to non-apoptotic nuclei. The extent of DNA fragmentation was quantitated by direct visual counting of green fluorescent labeled nuclei at 40× magnification. Five slides were examined per sample and apoptotic cells were scored out of a total of 100 cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

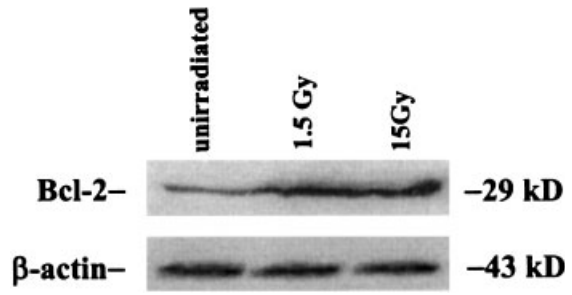

Fig. 3. Western blotting analysis of Bcl-2 expression in total homogenates deriving from Friend cells exposed to 1.5 and 15 Gy IR. Samples were normalized by goat β-actin polyclonal antibody incubation.

only a 1.1-fold increase in PKC ε expression and no significant changes in PKC δ expression were observed (Table I). These evidences led us to hypothesize a main involvement of PKC ζ and ε isoforms in Friend cells stress response and prompted us to investigate the specific in vitro PKC activity. A dose-dependent increased activity of total immunoprecipitated PKC ζ was observed upon 15 Gy, while PKC ε activity, although affected by radiation treatment, was not significantly modified (Fig. 5). Moreover, since the nuclear translocation of a molecule is often considered as a reasonable evidence of activation [Kelly et al., 1998], we performed Western blotting analysis on cytoplasmic and nuclear fractions.

Between the two isoforms tested, only PKC ζ was found significantly increased in nuclear extracts 1 h after irradiation (Fig. 6; Table II).

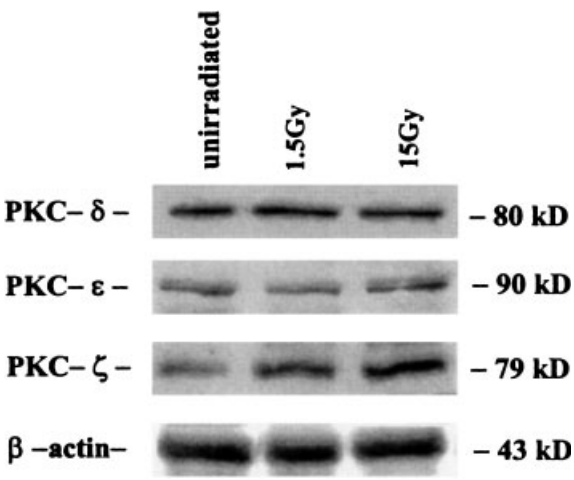

Fig. 4. Western blotting analysis of PKC δ, ε, and ζ expression in total homogenates deriving from Friend cells exposed to 1.5 and 15 Gy IR. Samples were normalized by goat β-actin polyclonal antibody incubation.

TABLE I. Densitometric Analysis of PKC δ, ε, and ζ Expression Detected by Western Blotting in Total Homogenates Derived From Friend Cells at 1 h After Irradiation

	PKC δ	PKC ε	PKC ζ
Unirradiated	1.33 ± 0.10	0.70 ± 0.06	0.73 ± 0.07*
1.5 Gy	1.36 ± 0.15	0.61 ± 0.04	1.16 ± 0.96
15 Gy	1.33 ± 0.18	0.77 ± 0.07	1.71 ± 0.85*

Data are the mean ± SD of five separate experiments.
*$P < 0.05$: 15 Gy PKC ζ versus unirradiated PKC ζ.

This observation was confirmed by immunoelectronmicroscopy semiquantitative "per cell" analysis which displayed a more abundant amount of PKC ζ within the cytoplasm of unirradiated cells (Table III), and a larger amount of protein within the nucleus of irradiated samples (Fig. 7), testifying for a PKC ζ nuclear translocation upon radiation exposure.

DISCUSSION

Protein Kinases C are important mediators both in apoptotic and survival responses switched on by IR in several cell lines [Haimovitz-Friedman, 1998; Yuan et al., 1998; Lee et al., 1999; Watters, 1999; Niehoff and Uckun, 2000]. Here, we report that exposure of Friend erythroleukemia cells to a high radiation dose determines changes in PKC ζ subcellular distribution and activation, which could sustain the low radiosensitivity of these cells. The selective involvement of this PKC isozyme was documented by in vitro specific activity assay, Western blotting, and immunoelectronmicroscopy techniques, which gave evidence to a dose-dependent increased activity and a nuclear

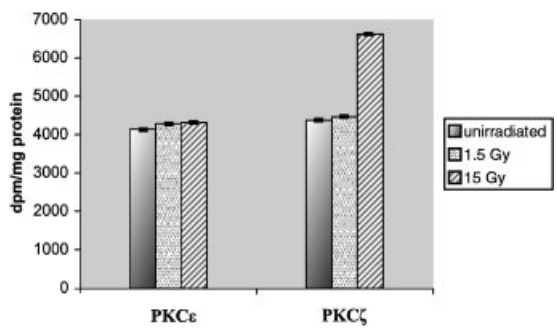

Fig. 5. In vitro specific PKC ε and ζ activities performed on immmunoprecipitated fractions derived from Friend cells at 1 h after irradiation. Values indicate dpm/mg protein. Data are the mean ± SD of three separate experiments *$P < 0.05$: 15 Gy PKC ζ versus unirradiated PKC ζ.

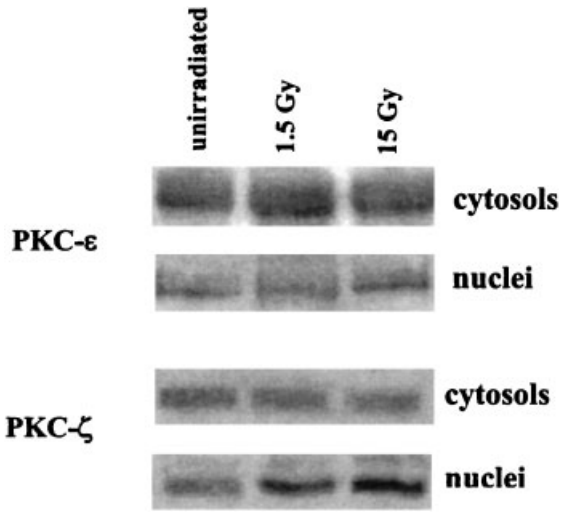

Fig. 6. Ionizing radiation induces a dose-dependent increase in nuclear PKC ζ expression in Friend cells. Each electrophoretic lane was loaded with an equal amount of protein (10 μg). The micrograph shows the most representative Western blotting out of five separate experiments.

localization of this isozyme, mainly due to the translocation from the cytoplasmic compartment and partly attributable to de novo protein synthesis. Cell growth and morphology were not greatly affected upon the low dose employed in this study (1.5 Gy), instead a dramatic inhibition of cell growth and an increased number of apoptotic and necrotic features were detected upon 15 Gy. Nevertheless, surviving Friend cells were able to proliferate in culture for long (not shown). Interestingly, a parallel and dose-dependent increase in Bcl-2 level was evidenced in total homogenates upon both IR doses, and this result is in accordance with the role suggested for the anti-apoptotic action of Bcl-2 during tumor progression observed in the same cell line [Howard et al., 2001]. In addition, in light of other reports suggesting a PKC/Bcl-2 interaction [Chen and Faller, 1995; Rinaudo et al., 1995; Deng et al., 2000; Kornblau et al., 2000], we can speculate that a possible mechanism through which PKC ζ modulates the survival of irradiated Friend cells implies modifications of the anti-apoptotic protein Bcl-2 expression. Presumably PKC ζ can functionally phosphorylate Bcl-2 at Ser 70 affecting, in turn, the stability of the interaction with its pro-apoptotic protein Bax, interaction which is thought to regulate cell survival [Yang and Korsmeyer, 1996]. This hypothesis is also consistent with previous findings by us and others, showing that when wortmannin, a specific inhibitor of PI-3-kinase and upstream regulator of PKC ζ [Le Good et al., 1998; Cataldi et al., 2002a], is added in culture before IR exposure, a higher number of apoptotic and dead cells is observed (26.7% in 15 Gy + WM vs. 22.2% in 15 Gy) and underlines the main role played by PKC ζ in mediating the occurrence of radioresistance in this specific cell line. The specificity of such a response is further supported by the evidence that in Jurkat T cells, another member of the PKC family, namely PKC δ, delivers an apoptotic signal mediating cytotoxic effects [Cataldi et al., 2002b]. Since previous results by our group evidenced the main role played by the pathway PI3K/AKT-1 in the maintenance of a survival signal in the same experimental model [Cataldi et al., 2001],

TABLE III. Analysis of PKC ζ-Bound Gold Granules Distribution in Friend Cells at 1 h After Ionizing Radiation Exposure

	Cytoplasms	Nuclei
Unirradiated	10.6 ± 0.7	12.1 ± 1.0*
1.5 Gy	10.8 ± 1.4	17.2 ± 1.3
15 Gy	17.2 ± 1.2	27.2 ± 1.8*

Data are the mean number ± SD of gold granules per μm^2 observed in 30 different fields at 20,000× magnification.
*$P < 0.05$: 15 Gy nuclei versus unirradiated nuclei.

TABLE II. Densitometric Analysis of PKC ζ and ε Expression Detected by Western Blotting in Cytoplasms and Nuclei Derived From Friend Cells at 1 h After Irradiation

	Cytoplasmic fraction		Nuclear fraction	
	PKC ε	PKC ζ	PKC ε	PKC ζ
Unirradiated	1.83 ± 0.15	1.90 ± 0.18	0.75 ± 0.06	0.87 ± 0.07*
1.5 Gy	1.97 ± 0.14	1.69 ± 0.17	0.70 ± 0.07	1.14 ± 0.09*
15 Gy	1.82 ± 0.17	1.44 ± 0.17	0.80 ± 0.06	1.91 ± 0.10*

Data are the mean ± SD of five separate experiments.
*$P < 0.05$: 15 Gy nuclear PKC ζ versus unirradiated nuclear PKC ζ and 1.5 Gy nuclear PKC ζ versus unirradiated nuclear PKC ζ.

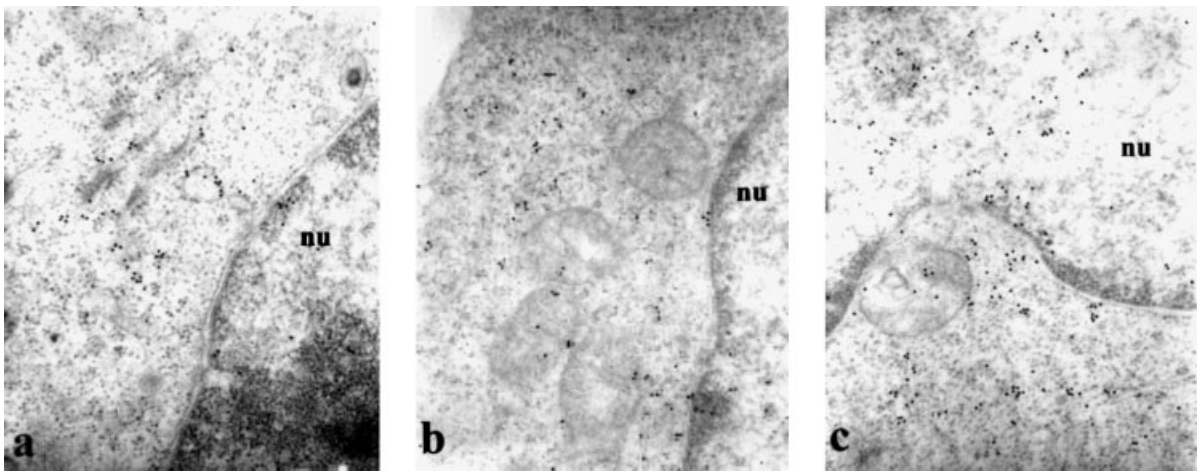

Fig. 7. Immunoelectronmicroscopy analysis of the subcellular distribution of PKC ζ in Friend erythroleukemia cells exposed to IR. Gold granules (15 nm) display the subcellular distribution of PKC ζ in the cytoplasm, around the nuclear envelope, at the nuclear pore and within the nucleus (20,000×). **a**: unirradiated; **b**: 1.5 Gy; **c**: 15 Gy.

further work is in progress to elucidate the existence of cross-talks between PI3K/AKT-1 and PKCs signaling pathways and the role of other Bcl-2 family members in determining the radioresistance of erythroleukemic cells. Anyway, altogether our results point at the recruitment of specific PKC isoforms in determining different cellular fates (death or survival) and indicate the PKC pathway as a possible target of therapeutic intervention in the radiation field.

REFERENCES

Buchner K. 1995. Protein kinase C in the transduction of signals toward and within the cell nucleus. Eur J Biochem 228:211–221.

Cataldi A, Di Pietro R, Centurione L, Grilli A, Cutroneo G, Miscia S. 2000. PI-3-kinase activation and atypical PKC ζ phosphorylation characterize the DMSO signalling in erythroleukemia cells. Cell Signal 12:667–672.

Cataldi A, Zauli G, Di Pietro R, Castorina S, Rana R. 2001. Involvement of the pathway phosphatidylinositol-3-kinase/AKT-1 in the establishment of the survival response to ionizing radiation. Cell Signal 13:369–375.

Cataldi A, Di Pietro R, Centurione L, Santavenere E, Garaci F, Rana R. 2002a. Ionizing radiation induces PI-3-kinase mediated activation and nuclear translocation of atypical PKC zeta. Cell Growth Differ (Submitted).

Cataldi A, Miscia S, Centurione L, Rapino M, Grifone G, Di Valerio V, Garaci F, Rana R. 2002b. Role of nuclear PKC δ in mediating caspase-3 up-regulation in Jurkat T leukemic cells exposed to ionizing radiation. J Cell Biochem 86: 553–560.

Chen YC, Faller DV. 1995. Direction of $p21^{ras}$ generated signal towards cell growth or apoptosis is determined by Protein Kinase C and Bcl-2. Oncogene 11:1487–1492.

Chinnayian AM, Prasad U, Shankar S, Hamstra DA, Shanaiah M, Chenevert TL, Ross BD, Rehemtulla A. 2000. Combined effect of tumor necrosis factor related apoptosis-inducing ligand and ionizing radiation in breast cancer therapy. Proc Natl Acad Sci USA 97: 1754–1759.

Cross T, Griffiths G, Deacon E, Sallis R, Gough M, Watters D, Lord JM. 2000. PKC δ is an apoptotic lamin kinase. Oncogene 4 19(19):2331–2337.

Deng X, Ruvolo P, Carr B, Stratford May, Jr. 2000. Survival function of ERK1/2 as IL-3 activated, staurosporine-resistant Bcl-2 kinases. PNAS 97(4):1578–1583.

Di Pietro R, Secchiero P, Rana R, Gibellini D, Visani G, Bernis K, Zamai L, Miscia S, Zauli G. 2001. Ionizing radiation sensitizes erythroleukemic cells but not normal erythroblasts to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated cytotoxicity by selective up-regulation of TRAIL-R1. Blood 97(9):2596–2603.

Einspanner M, Boulton JE, Borsa J. 1984. Variation of radiation sensitivity of Friend erythroleukemia cultured in the presence of the differentiation inducer DMSO. Radiat Res 97(1):55–63.

Haimovitz-Friedman A. 1998. Radiation-induced signal transduction and stress response. Radiat Res 150(5): S102–S108.

Howard JC, Li Q, Chu W, Zochodne B, Kapoor M, Ung Y, Rosen K, Ben-David Y. 2001. Bcl-2 expression in F-MuLV-induced erythroleukemias: A role for the anti-apoptotic action of Bcl-2 during tumor progression. Oncogene 20(18):2291–2300.

Keeman C, Kelleher D. 1998. Protein kinase C and the cytoskeleton. Cell Signal 10:225–232.

Kelly ML, Tang Y, Rosensweigh N, Clejan S, Beckman BS. 1998. Granulocyte-macrophage colony stimulating factor rescues TF-1 leukemia cells from ionizing radiation-induced apoptosis through a pathway mediated by PKC α. Blood 92(2):416–424.

Kornblau SM, Vu HT, Ruvolo P, Estrov Z, O'Brien S, Cortes J, Ka H, Andreeff M, May WS. 2000. Bax and PKC alpha modulate the prognostic impact of Bcl-2 expression in acute myelogenous leukemia. Clin Cancer Res 6(4): 1401–1409.

La Porta CA, Tessitore L, Comolli R. 1997. Changes in PKC α, δ and in nuclear β isoform expression in tumor and lung metastatic nodules induced by diethylnitrosamine in the rat. Carcinogenesis 18:715–719.

Lavin MF, Watters D, Song Q. 1996. Role of protein kinase activity in apoptosis. Experientia 52(10–11):979–994.

Le Good JA, Ziegler WH, Parekh DB, Alessi DR, Cohen P, Parker PJ. 1998. Protein Kinase C isotypes controlled by phosphoinositide-3-kinase through Protein kinase PDK-1. Science 281:2042–2045.

Lee SI, Cho CK, Yoo SY, Kim TH, Lee YS. 1999. Influence of ionizing radiation on induction of apoptotic cell death and cellular redistribution of PKC isozymes in mouse epidermal cells differing in carcinogenesis stages. Mutat Res 426(1):41–49.

Mallia CM, Aguirre V, Mc Gary E, Tang Y, Scandurro AB, Liu C, Noguchi CT, Beckman BS. 1999. Protein kinase C α is an effector of hexamethylenebisacetamide induced differentiation in Friend erythroleukemia cells. Exp Cell Res 246:348–354.

Martelli AM, Sang N, Borgatti P, Capitani S, Neri LM. 1999. Multiple biological responses activated by nuclear PKC. J Cell Biochem 74:499–521.

Mellor H, Parker PJ. 1998. The extended protein kinase C superfamily. Biochem J 332:281–292.

Nakanishi H, Brewer KA, Exton JH. 1993. Activation of the ζ isozyme of PKC by PI3,4,5P3. J Biol Chem 268(1):13–16.

Negoescu A, Lorimier P, Labat-Moleur F, Drouet C, Robert C, Guillermet C, Brambilla C, Brambilla E. 1996. In situ apoptotic cell labeling by the TUNEL method: Improvement and evaluation on cell preparations. J Histochem Cytochem 44(9):959–968.

Newton AC. 1997. Regulation of protein kinase C. Curr Opin 9:161–167.

Niehoff LB, Uckun FM. 2000. Role of PKC μ in radiation-induced increase of JUN protoncogene mRNA levels in B lineage lymphoid cells. Radiat Res 154(2):145–150.

Olson EN, Burgess R, Standinger J. 1993. Protein kinase C as a transducer of nuclear signals. Cell Growth Differ 4:699–705.

Rinaudo MS, Su K, Falk LA, Haldar S, Mufson RA. 1995. Human interleukin-3 receptor modulates bcl2 mRNA and protein levels through Protein Kinase C in TF-1 cells. Blood 86:80–85.

Sajan MP, Standaert ML, Bandyopadhyay G, Quon MJ, Burke TR, Jr., Farese RV. 1999. PKC ζ and PDK1 are required for insulin-induced activation of ERK in rat adipocytes. J Biol Chem 274:30495–30500.

Shimizu T, Cao CX, Shao RG, Pommier Y. 1998. Lamin B phosphorylation by PKC α and proteolysis during apoptosis in human leukemia HL60 cells. J Biol Chem 273: 8669–8674.

Takeda H, Matoraki T, Takada T, Noguchi T, Yamao T, Tsuda M, Ochi F, Fukunaga K, Inagaki K, Kasuga M. 1999. PI3K γ and PKC ζ mediate ras independent activation of MAP kinase by a G protein coupled receptor. EMBO J 18:386–395.

Watters D. 1999. Molecular mechanisms of ionizing radiations induced apoptosis. Immunol Cell Biol 77(3):263–271.

Yang E, Korsmeyer SJ. 1996. Molecular thanatopsis: A discourse on the Bcl-2 family and cell death. Blood 88: 386–401.

Yuan ZM, Utsugisawa T, Ishiko T, Nakada S, Huang Y, Kharbanda S, Weichselbaum R, Kufe D. 1998. Activation of PKC δ by the c-Abl tyrosine kinase in response to ionizing radiation. Oncogene 16(13):1643–1648.