Intranucleolar Localization of DNA Topoisomerase IIα Is a Distinctive Feature of Necrotic, But Not of Apoptotic, Jurkat T-Cells

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ABSTRACT Two distinct types of cell death have been described: apoptosis and necrosis. However, it is becoming increasingly clear that the differences between these two types are far less numerous than initially thought. Morphological analyses might provide important information to distinguish apoptotic from necrotic samples. We recently reported that in necrotic, but not apoptotic, HL-60 human myeloid leukaemia cells, the nuclear protein topoisomerase IIα concentrated in nucleoli. In order to ascertain whether or not this phenomenon was restricted to a peculiar cell type or could be detected also in cells of lymphoid lineage, we performed an investigation aimed at defining the localization of topoisomerase IIα in apoptotic and necrotic Jurkat human T lymphoblastoid cells. Immunofluorescence staining demonstrated that topoisomerase IIα was excluded from the condensed chromatin of apoptotic cells, whereas in necrotic cells it was localized in discrete nuclear dots. Immuno-electron microscopy analysis showed that topoisomerase IIα was undetectable in nucleoli of normal and apoptotic cells, whereas it was present in the nucleolus of necrotic cells irrespectively of the type of inducer used (ethanol, H_2O_2, $HgCl_2$). Taken together, our findings identify topoisomerase IIα as a potential morphological marker useful to discriminate between apoptotic and necrotic cells. *Microsc. Res. Tech. 62:192–200, 2003.*

INTRODUCTION

Cell death is said to occur by two alternative, opposite modes: apoptosis, a programmed form of cell death, and necrosis, an unordered and accidental form of cellular dying (Buja et al., 1993; Majno and Joris, 1995). The term apoptosis, defined as a controlled type of cell death that can be induced by a plethora of physiologic and pharmacological treatments, was first employed by Kerr et al. (1972) mainly on the basis of morphological criteria: cell shrinkage, condensation and margination of chromatin, cytoplasmic vacuolization, and final cell splitting in a number of "apoptotic bodies." Subsequently, many biochemical changes have been recognized to occur during apoptosis, chiefs of which are DNA fragmentation and protein cleavage (Hengartner, 2000). Necrosis is characterized by nuclear pyknosis, cytoplasmic swelling, and a progressive loss of membrane integrity (Wyllie et al., 1980). An extensive body of literature exists on the apparent differences between apoptosis and necrosis (reviewed in Kanduc et al., 2002). For example, while apoptosis is an energy-dependent method of cellular deletion without inflammation, necrosis is associated with inflammation; apoptosis is the consequence of mild injuries whereas necrosis occurs in response to more severe forms of the same types of injury.

More recently, however, several investigations have highlighted the fact that the differences between necrosis and apoptosis might be less numerous than initially believed. Indeed, some of the biochemical changes that take place during apoptosis have been demonstrated to occur also during necrosis: the inner mitochondrial membrane may become permeable to ions and small molecules during both necrosis and apoptosis (Dynlacht et al., 1999). Furthermore, the activation of cysteine proteases and the cleavage of poly-(ADP-ribose) polymerase and other proteins have been documented to occur during both modes of cell death (Casiano et al., 1998; Shah et al., 1996). The differences in the protein cleavage pattern of apoptotic and necrotic cells might be important for a differential diag-

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nosis between these two conditions (Casiano et al., 1998).

However, it has been suggested that morphological criteria might also be an effective tool to distinguish apoptotic from necrotic changes (Kanduc et al., 2002).

We recently demonstrated that in necrotic, but not in apoptotic, HL-60 human leukemia cells, the distribution of the abundant nuclear protein DNA topoisomerase IIα, underwent dramatic changes consisting of the appearance of large and bright dots apparently localized to nucleoli (Bortul et al. 2001). These findings suggested that the distribution of DNA topoisomerase IIα might be considered, in principle, a differential morphological criterion of necrotic cell death. However, it remained to be established that such a redistribution was not restricted to HL-60 cells that underwent necrotic death following exposure to ethanol.

In this study, we demonstrate that a similar redistribution of DNA topoisomerase IIα localization takes place in Jurkat human T leukemia cells treated with different necrosis inducers. Indeed, by immuno-electron microscopy we detected topoisomerase IIα in nucleoli of necrotic cells.

Our results seem to indicate that a change in the spatial distribution pattern of topoisomerase IIα is a consistent feature of necrotic cell death and could, therefore, be used as a morphological criterion to differentiate apoptotic from necrotic cells.

MATERIALS AND METHODS

Cell Culture and Induction of Apoptosis or Necrosis

Jurkat human T cells were grown in RPMI 1640 medium containing 10% fetal calf serum at 37°C in a humified atmosphere, and passaged every 3 days by 5-fold dilution into fresh medium. For induction of apoptosis with antibody to Fas (CD95/APO-1), cells were washed twice in serum-free medium before the addition of 200 ng/ml of monoclonal antibody CH-11 (Medical and Biological Laboratories, Watertown, MA). For treatment with camptothecin (5 μM) or etoposide (150 μM), the drugs were added to cells growing in the complete medium. To induce necrosis, cells were treated with 40 μM $HgCl_2$, or 10% ethanol, or 0.1% H_2O_2 (Casiano et al., 1998). All treatments inducing apoptosis or necrosis were performed for 7 hours, unless otherwise indicated.

Flow Cytometry

For identification and quantification of apoptosis, cells were fixed with 70% ethanol at 4°C for 30 minutes. Samples were stained with 50 μg/ml propidium iodide (PI) and 50 μl of a 1 mg/ml RNase A solution. They were then incubated at 37°C for 30 minutes and analyzed by a FACscan (Becton Dickinson, Palo Alto, CA) equipped with an argon ion laser tuned at 488 nm to excite PI. PI red fluorescence was collected on a linear scale and apoptosis was identified as a subdiploid area. To analyze necrosis, the Annexin V-7 amino-actinomycin D (7-AAD) staining kit (Beckman-Coulter, Miami, FL) was employed. Annexin V, conjugated with fluorescein isothiocyanate (FITC), binds to cells expressing phosphatidylserine on the outer layer of the plasma membrane while 7-AAD stains DNA of cells with a damaged plasma membrane. This allows discrimination of undamaged cells (unstained with either fluorochrome) from apoptotic cells (stained only with Annexin V-FITC) or necrotic cells (stained with both Annexin V-FITC and 7-AAD).

DNA Gel Electrophoresis

DNA was extracted using standard procedures (Falcieri et al., 2000). Five micrograms DNA/lane were loaded on 1.8% agarose gel in Tris-borate/EDTA buffer, stained with 0.5 μg/ml ethidium bromide, and visualized on a UV transilluminator.

Conventional Transmission Electron Microscopy (TEM)

Cells were fixed for 1 hour at room temperature with 2.5% glutaraldehyde buffered in phosphate buffer, postfixed in 1% OsO_4 in the same buffer, dehydrated in a graded ethanol series, and embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate and observed using a Philips CM 10 electron microscope (Falcieri et al., 2000). In some cases, samples were processed as for immuno-electron microscopy (Luchetti et al., 2002).

Polyacrylamide Gel Electrophoresis and Immunoblotting of Cell Lysates

Cells were sedimented at 1,000*g* for 10 minutes and washed twice in phosphate buffered saline (PBS) containing the COMPLETE Protease Inhibitor Cocktail (Roche Applied Science), according to the manufacturer's instructions. Cells were then resuspended at $\sim 10^7$/ml in lysis buffer containing 62.5 mM Tris-HCl, pH 6.8, 2% sodium dodecylsulfate (SDS), 10% glycerol, 5% 2-mercaptoethanol, and the protease inhibitor cocktail. Lysates were boiled for 5 minutes to solubilize protein, briefly sonicated to shear DNA, and stored at −80°C until required. Cell harvesting and lysate preparation were conducted in the presence of the protease inhibitor cocktail as a precaution to prevent further proteolysis. Total protein from $\sim 1 \times 10^6$ cells was separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred to nitrocellulose sheets using a semi-dry blotting apparatus. Sheets were saturated in PBS containing 5% normal goat serum (NGS) and 4% bovine serum albumin (BSA) for 60 minutes at 37°C (blocking buffer), then incubated overnight at 4°C in blocking buffer containing the monoclonal antibody to topoisomerase IIα(Roche Applied Science) diluted 1:500. After four washes in PBS containing 0.1% Tween 20, the sheets were incubated for 30 minutes at room temperature with peroxidase-conjugated anti-mouse IgG (Sigma Chemical Co., St Louis, MO), diluted 1:5,000 in PBS-Tween 20, and washed as above. Bands were visualized by the enhanced chemiluminescence (ECL) method using Lumi-Light^{Plus} (Roche Applied Science).

Protein Assay

Assays were performed as described by Bradford (1976).

Immunofluorescent Staining

Cells in media were plated onto 0.1% poly-L-lysine-coated glass slides and adhesion was allowed to proceed for 30 minutes at room temperature. Samples

were fixed in freshly-prepared 4% paraformaldehyde in PBS for 30 minutes at room temperature and then permeabilized with 0.2% Triton X-100 for 10 minutes. After several washes with PBS, nonspecific binding of antibodies was blocked by a 30-minute incubation at 37°C with PBS, 2% BSA, 5% NGS. Slides were then incubated for 3 hours at 37°C with the antibody to topoisomerase IIα (diluted 1:50) in PBS, 2% BSA, 5% NGS. Slides were then washed three times in PBS and reacted with FITC-conjugated anti-mouse IgG (from Sigma), diluted 1:200 in PBS, 2% BSA, 5% NGS for 1 hour at 37°C. Samples were subsequently washed three times in PBS, stained with 0.01 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) in PBS and mounted in 20 mM Tris-HCl, pH 8.2, 90% glycerol containing 2.3% of the antifading agent 1,4-diazobicyclo-(2.2.2)-octane. Slides were observed and photographed using a Zeiss Axiophot epifluorescence microscope.

Immunogold Labeling and Electron Microscopy

Cells were fixed with 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 1 hour and washed twice in phosphate buffer. Samples were partially dehydrated up to 70% ethanol and embedded in London Resin White (LRW). Thin sections were collected on 400-mesh nickel grids and incubated for 30 minutes at room temperature in TBS blocking buffer (50 mM Tris-HCl, pH 7.6, 1% sodium azide, 150 mM NaCl, 0.1% BSA). The grids were treated with 5% NGS for 30 minutes at room temperature, and incubated overnight at 4°C in the presence of the antibody to topoisomerase IIα, used at 1:100 dilution in blocking buffer. After several washes in TBS buffer, the samples were incubated in TBS buffer (pH 8.2) for 1 hour at room temperature with 10-nm gold-conjugated goat anti-mouse IgG (British Biocell International, Cardiff, UK) diluted 1:100 in blocking buffer. After three washes in TBS (pH 8.2) and one in distilled water, the grids were counterstained with uranyl acetate and lead citrate. Controls were represented by samples treated with the secondary antibody alone: no gold particles were detected (data not shown). All samples were examined with a Philips CM 10 electron microscope operating at 80 kV (Luchetti et al., 2002).

RESULTS

Flow Cytometric Analysis

We first evaluated by means of flow cytometry the efficacy of our inducers in causing apoptotic or necrotic cell death. Apoptotic cells were identified as a subdiploid peak on the basis of PI staining, while necrotic cells were distinguished using a combined Annexin V-FITC/7-AAD staining. As shown in Figure 1, analysis by flow cytometry showed that treatment with anti-FAS, camptothecin, or etoposide resulted in a similar percentage of apoptotic Jurkat cells, which ranged between 72 and 80%. The percentage of necrotic cells in response to these treatments was very low (around 3–4%). Ethanol, H_2O_2 or $HgCl_2$ were equally effective in inducing Jurkat T cell death by necrosis, in a percentage which ranged between 83 and 91%. These treatments caused apoptosis only in 4–5% of cells. Figure 2A shows that apoptotic cells were indeed stained by Annexin V but not by 7-AAD, as expected, and a subdiploid area was obtained by cell cycle analysis of PI-stained samples. In contrast, necrotic cells were 7-AAD positive and Annexin V positive, and no subdiploid area was seen upon cell cycle analysis (Fig. 2B).

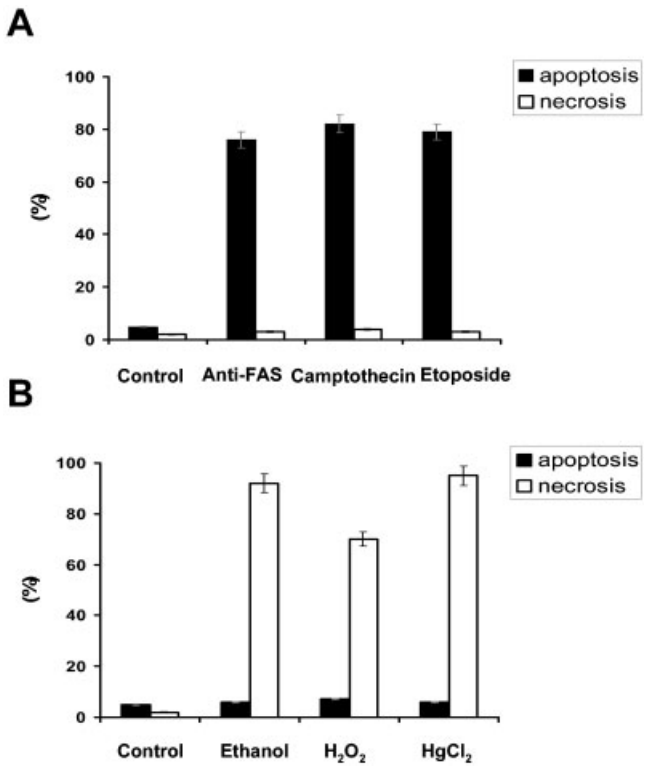


Fig. 1. Flow cytometric analysis of Jurkat T cells treated for 7 hours with agents producing apoptosis or necrosis. Results are the mean ± SD from three separate experiments and show the percentage of apoptotic or necrotic cells in response to the different inducers. **A:** Treatment with apoptosis inducers. **B:** Treatment with necrosis inducers.

DNA Gel Electrophoresis

As a further control, we performed DNA gel electrophoresis. As shown in Figure 3, in camptothecin-treated samples we detected the characteristic internucleosomal DNA fragmentation. Similar results were seen in samples exposed to anti-FAS or etoposide (not shown). In contrast, no DNA fragmentation was visible in samples treated with ethanol. Similar results were observed following exposure to either H_2O_2 or $HgCl_2$ (not shown).

Conventional TEM

Jurkat cells, when examined at TEM, showed a large nucleus, with a prominent nucleolus, and a thin cytoplasmic layer with a scarce organellar component (Fig. 4A). When triggered to apoptosis with camptothecin, the well-known nuclear changes appeared such as electron-dense micronuclei (Fig. 4B). It is interesting that some micronuclei, as the one presented in Figure 4B, did not display a surrounding membrane. Usually, the majority of micronuclei appear membrane-surrounded even if, rarely, they are not (Columbaro et al., 1998). In this case, the fate of the nuclear envelope has been suggested to be "annulatae lamellae," i.e., unusual

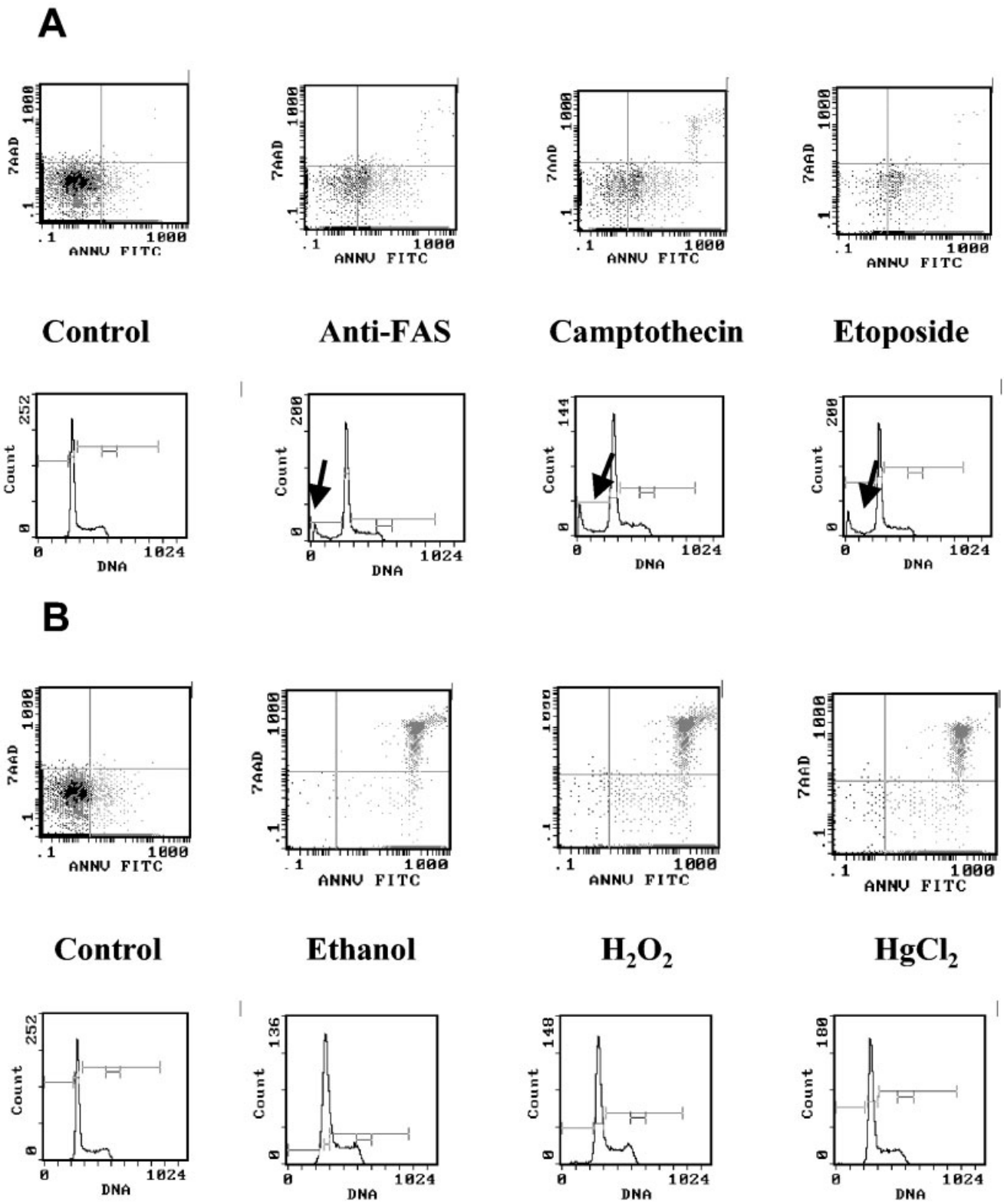


Fig. 2. Representative histograms of apoptotic cells (**A**) or necrotic cells (**B**) analyzed by flow cytometry. **Top panels:** Annexin V vs. 7-ADD (apoptotic cells are Annexin V +/7-AAD-; necrotic cells are Annexix V-/7-AAD +). **Bottom panels:** Cell cycle staining by PI: apoptotic cells are identified in the pre G_0/G_1 area (subdiploid area indicated by the arrows). Note that samples treated with necrosis inducers do not display cells in the pre G_0/G_1 area (subdiploid area). Cells have been treated with the inducers for 7 hours.

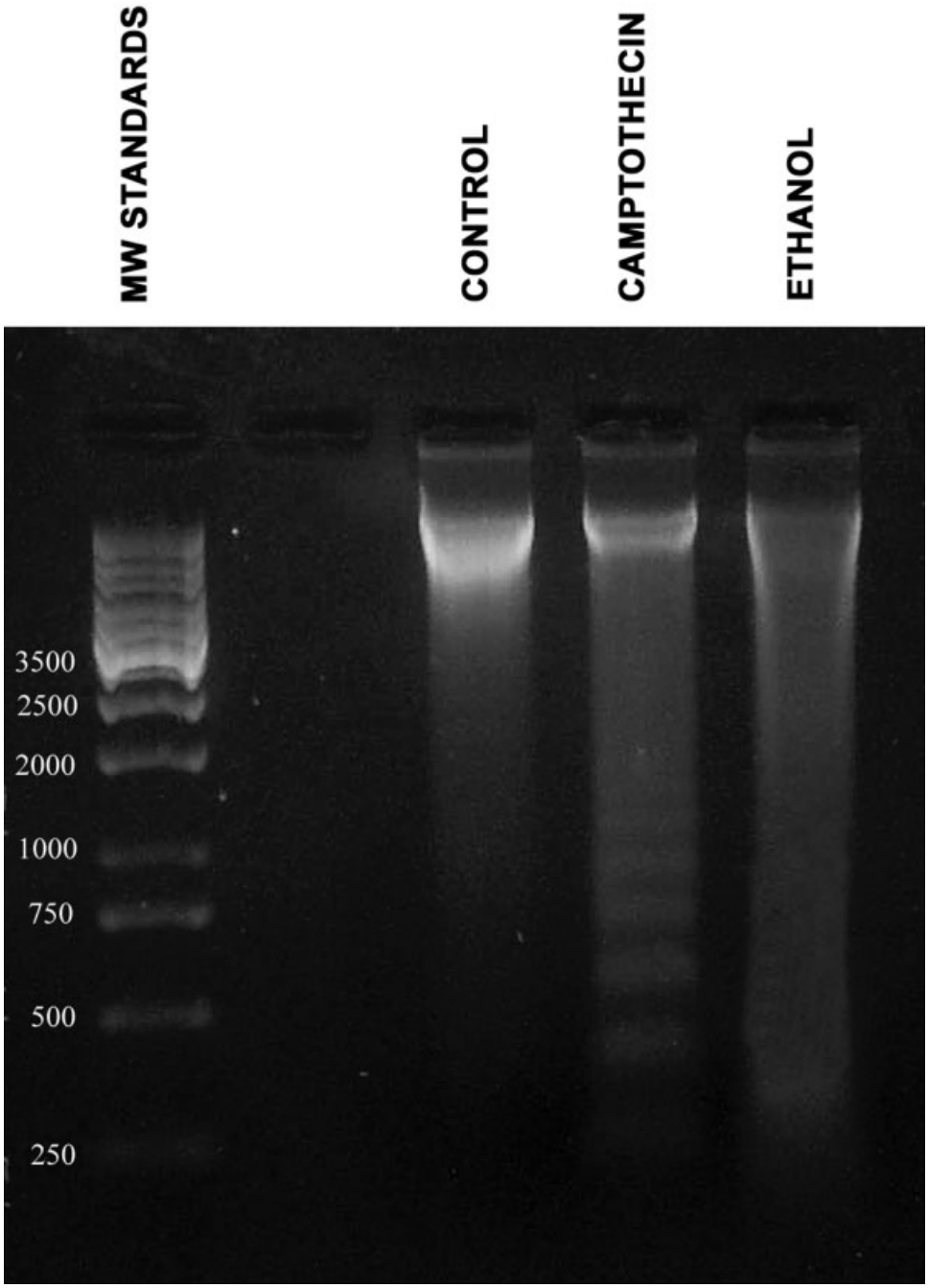


Fig. 3. DNA gel electrophoresis from control, camptothecin, and ethanol treated Jurkat T cells. Molecular weight markers are at left.

structures formed by flattened cisternae consisting of stacks of nuclear pore-containing membranes that have been described occasionally in apoptosis (Falcieri et al., 1994). Necrotic death, induced by a 7-hour treatment with 10% ethanol, even if in the presence of a general cell swelling and disruption, allowed a satisfactory preservation of overall nuclear structure. This was evident after 2.5% glutaraldehyde conventional fixation, alcohol dehydration, and epoxy resin embedding (Fig. 4C). Surprisingly, nuclear components were recognizable also after a mild fixation, a partial dehydration, and London Resin White embedding, i.e., the procedure required for immuno-electron microscopy (Fig. 4D).

Western Blot Analysis

We next analyzed by Western blot the possible cleavage of topoisomerase IIα in apoptotic or necrotic samples. As shown in Figure 5, topoisomerase IIα was cleaved after treatment with anti-FAS, camptothecin, or etoposide. In contrast, no cleavage at all was detected following exposure to ethanol, H_2O_2 or $HgCl_2$.

Immunofluorescence Staining

The antibody to topoisomerase IIα gave a diffuse fluorescent pattern when employed to immunostain nuclei of control Jurkat cells (Fig. 6A). In apoptotic

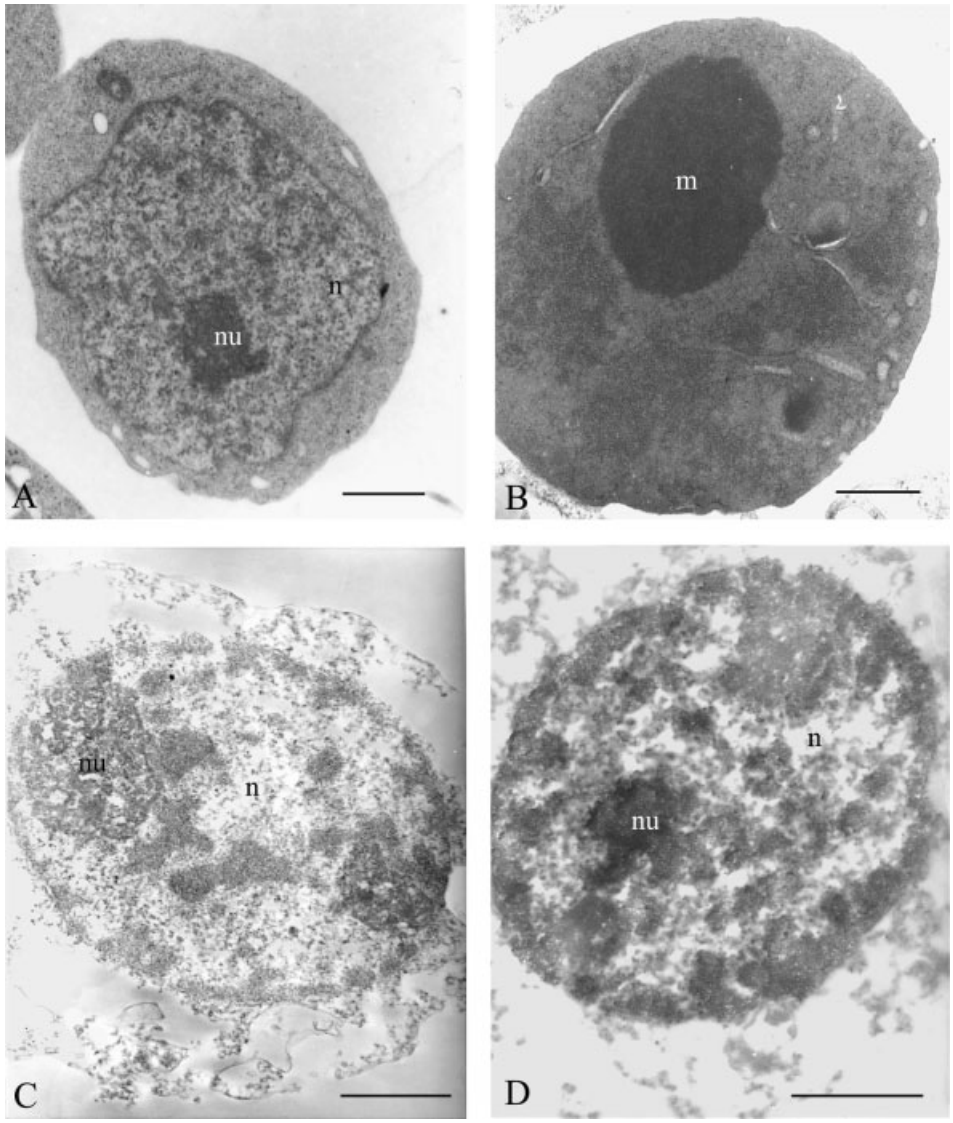


Fig. 4. Conventional TEM analyses of Jurkat T cell. **A**: Araldite-embedded control cell; **B,C**: camptothecin-and ethanol treated Araldite-embedded cells, respectively; **D**: LRW-embedded control cell. n: nucleus; nu: nucleolus; m: micronucleus. Scale bar: 1 μm.

samples, the immunoreactivity was excluded from the area of condensed chromatin revealed by DAPI staining (Fig. 6C–H). In necrotic samples, besides a diffuse nucleoplasmic positivity, we detected 3–5 large fluorescent dots per nucleus (Fig. 6I–N). This suggested that topoisomerase IIα could be concentrated in nucleoli.

Immuno-Electron Microscopy Analysis

In order to identify the nuclear domains of necrotic Jurkat cells in which topoisomerase IIα concentrated, we performed immuno-electron microscopy analysis. The nucleoli of control cells were unlabeled (Fig. 7A). Anti-topoisomerase IIα immunolabelling was then performed in cells undergoing necrosis by means of ethanol (Fig. 7B), $HgCl_2$ (Fig. 7C) or H_2O_2 (Fig. 7D). In all of the three conditions, gold particles appeared mainly localized within the nucleoli, mostly on their granular component. In contrast, in nucleoli of apoptotic cells treated with anti-FAS antibody for 2 hours (i.e., a time frame in which nucleoli were still identifiable), nucleoli were negative for topoisomerase IIα (Fig. 7E). Similar results were observed when either camptothecin or etoposide were employed to induce apoptosis (not shown).

DISCUSSION

In this study, we have reported the results of an investigation designed to analyze whether the changes in the localization of topoisomerase IIα previously described in HL-60 necrotic cells could also be detected in Jurkat T human leukemia cells treated with three different necrosis inducers.

We first performed control experiments employing flow cytometry analysis, DNA gel electrophoresis, and TEM in order to verify that we dealt with populations that were highly enriched in either apoptotic or necrotic cells. We incubated cells for 7 hours with the inducers because we found that shorter treatments did not allow a reproducible identification of the apoptotic ultrastructural features. On the other hand, longer

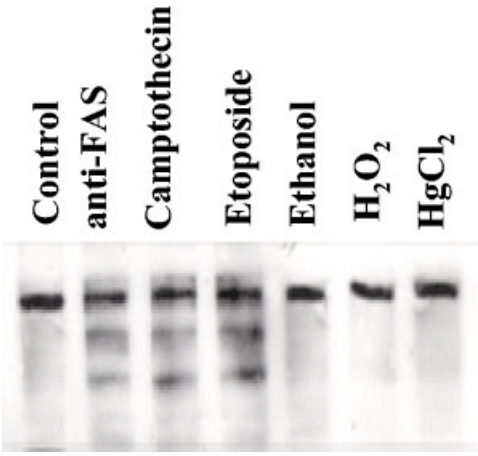


Fig. 5. Western blotting analysis for topoisomerase IIα during apoptosis or necrosis in Jurkat T cells. Cells (1×10^7) were lysed in 1 ml of electrophoresis sample buffer, briefly sonicated, and boiled. Protein was separated by SDS-PAGE and transferred to nitrocellulose sheets that were then probed with a monoclonal antibody. Protein (80 μg) was blotted to each lane. Bands were visualized by the ECL method. The gel is representative of three separate experiments.

incubation times induced in necrotic samples a general cell disruption, which made it impossible to recognize cell components.

Since we recently reported that topoisomerase IIα was not proteolytically cleaved in HL-60 human leukemia cells undergoing necrosis by ethanol treatment (Bortul et al., 2001), we wanted to analyze whether or not this was also true of Jurkat T cells. Western blot analysis confirmed that topoisomerase IIα was not cleaved after exposure to necrosis inducers, whereas it was degraded to multiple fragments in apoptotic cells. However, we could not rule out that the antibody we used could not recognize different fragments present in necrotic samples only, even though it should be emphasized that the amount of the native topoisomerase IIα did not decrease in necrotic samples so that a cleavage seems unlikely. In any case, Casiano et al. (1998) reported also that in necrotic Jurkat T cells, topoisomerase IIα was essentially uncleaved.

Immunofluorescence staining for topoisomerase IIα showed that in apoptotic cells, it was always excluded from the areas of compact chromatin. This is in agreement with a previous study carried out in HL-60 cells (Sugimoto et al., 1998).

In contrast, in necrotic Jurkat cells we detected in each nucleus several brilliant and large dots, besides a more diffuse staining. Immuno-electron microscopy allowed us to conclude that the nucleoli of necrotic cells were labeled by the antibody to topoisomerase IIα. On the other hand, neither control nor apoptotic cells showed any immunolabeling in nucleoli. In this context, it should be emphasized that the nucleolus is one of the nuclear domains that are more resistant to the

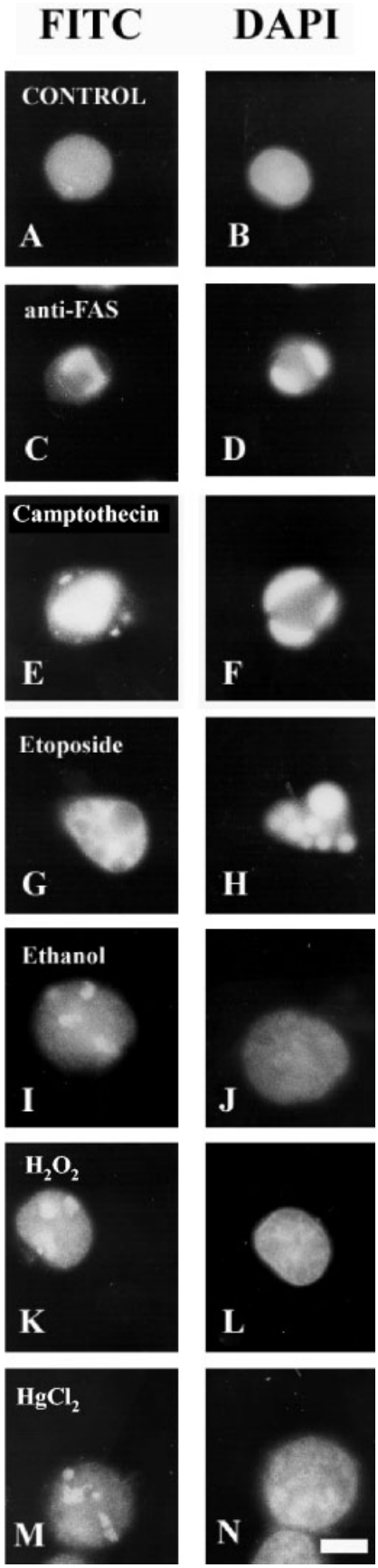
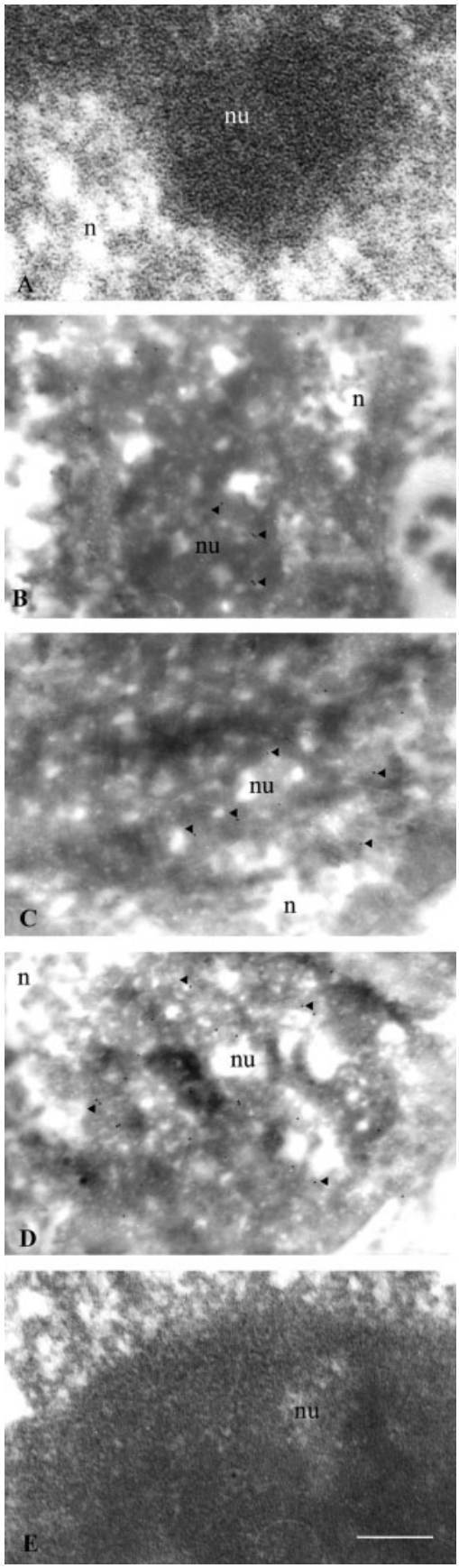


Fig. 6. Immunofluorescence staining of control, apoptotic, and necrotic Jurkat T cells. Scale bar: 5 μm.



apoptotic process (Martelli et al., 1999a,b). By immunoelectron microscopy, some gold particles were also detected in the nucleoplasm of necrotic cells and this is in agreement with the results of the immunofluorescence staining. From these data and those of Western blotting experiments, it is deduced that in both necrotic and apoptotic cells there is a non-nucleolar localization of topoisomerase IIα that is in the form of fragments for the apoptotic cells and uncleaved for the necrotic. At present, we do not know the physiological meaning for this.

Human topoisomerase IIα, once thought to be an immobile, structural component of the chromosomal scaffold or the interphase nucleoskeleton, has recently been shown to be a very dynamic interaction partner of these structures. Indeed, an investigation carried out by means of stable overexpression of a chimera consisting of topoisomerase IIα tagged with green fluorescent protein, showed a dynamic behavior of the enzyme during mitosis, the interphase, or in response to drug treatment (Christensen et al., 2002). What might be the functional significance of the intranucleolar topoisomerase IIα in necrotic cells? It has been proposed that topoisomerase IIα is involved in DNA repair (e.g., Giocanti et al., 1993; Thielmann and Popanda, 1998). On the other hand, agents that produce necrosis, such as ethanol and H_20_2, cause DNA damage (Lee et al., 2002; Russo et al., 2001). We could, therefore, hypothesize that the intranucleolar presence of topoisomerase IIα might be related to DNA repair processes potentially taking place in necrotic cells, even though energy-consuming activities such as DNA repair have yet not been demonstrated to take place during necrosis.

The localization of topoisomerase IIα in the nucleolus of healthy cells is still contentious. For several years, the nucleolus-specific isoform of topoisomerase II was considered to be the β (Govoni et al., 1995; Zini et al., 1992). Subsequently, however, this conclusion has been questioned (Chaly and Brown, 1996). Other investigators have shown that topoisomerase IIα was also detectable in nucleoli (Christensen et al., 2002; Zini et al., 1994). However, our results did not show the presence of topoisomerase IIα in nucleoli of healthy Jurkat T cells.

We would like to emphasize that HL-60 and Jurkat cell lines, although both of hematopoietic lineage, are different, since the former is of myeloid origin whereas the latter is of lymphoid derivation. Conceivably, the changes in the distribution pattern of topoisomerase IIα are to be considered a consistent feature of necrotic cells. In light of these findings, we propose that the differential immunostaining pattern for topoisomerase IIα we have identified may be very useful in the future for allowing a differential diagnosis between necrotic and apoptotic samples, especially in the context of pathological processes in vivo, which has never been an easy task if ultrastructural analysis could not be em-

Fig. 7. Immuno-electron microscopy analysis of control Jurkat T cells (**A**), of cells after necrotic death induction by ethanol (**B**), $HgCl_2$ (**C**), H_2O_2 (**D**), and of apoptotic cells treated with anti-FAS antibody for 2 hours. Gold particles (arrowheads) immunolabeling topoisomerase IIα appear mostly localized in nucleolar granular component. n: nucleus; nu: nucleolus. Scale bar: 0.2 μm.

ployed (Columbano, 1995; Farber, 1994; Majno and Joris, 1995). It might also be a useful tool to discriminate between apoptosis and necrosis in particular conditions such as the very late stages of both processes where a general cell disruption often occurs. In contrast, early necrotic and apoptotic features are generally well distinguishable: therefore, nucleolar localization of topoisomerase IIα, if already detectable, could only be considered an additional discriminating feature.

Moreover, future investigations should clarify whether nucleolar localization of topoisomerase IIα could represent a further criterion to characterize cell death, especially when considering recently identified phenomena such as aponecrosis (Formigli et al., 2000) and anoikis (Grossmann, 2002).

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REFERENCES

Bortul R, Zweyer M, Billi AM, Tabellini G, Ochs RL, Bareggi R, Cocco L, Martelli AM. 2001. 2001. Nuclear changes in necrotic HL-60 cells. J Cell Biochem 81:19–31.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Buja LM, Eigenbrodt ML, Eigenbrodt EH. 1993. Apoptosis and necrosis: basic types and mechanisms of cell death. Arch Pathol Lab Med 117:1208–1214.

Casiano CA, Ochs RL, Tan EM. 1998. Distinct cleavage products of nuclear proteins in apoptosis and necrosis revealed by autoantibody probes. Cell Death Differ 5:183–190.

Chaly N, Brown DL. 1996. Is DNA topoisomerase IIβ a nucleolar protein? J Cell Biochem 63:162–173.

Christensen MO, Larsen MK, Barthelmes HU, Hock R, Andersen CL, Kjeldsen E, Knudsen BR, Wetergaard O, Boege F, Mielke C. 2002. Dynamics of human DNA topoisomerase II α and IIβ in living cells. J Cell Biol 157:31–44.

Columbano A. 1995. Cell death: current difficulties in discriminating apoptosis from necrosis in the context of pathological processes in vivo. J Cell Biochem 58:181–190.

Columbaro M, Gobbi P, Renò F, Lucheti F, Santi S, Falcieri E. 1998. Different technical approaches to the study of apoptotic micronuclei. Scanning 20:248–249.

Dynlacht JR, Earles M, Henthorn J, Roberts ZV, Howard EW, Seno JD, Sparling D, Story MD. 1999. Degradation of the nuclear matrix is a common element during radiation-induced apoptosis and necrosis. Radiat Res 152:590–603.

Falcieri E, Gobbi P, Castaldi A, Zamai L, Feanza I, Vitale M. 1994. Nuclear pores in the apoptotic cell. Histochem J 26:754–763.

Falcieri E, Luchetti F, Burattini S, Canonico B, Santi S, Papa S. 2000. Lineage-related sensitivity to apotosis in human tumor cells undergoing hyperthermia. Histochem Cell Biol 113:135–144.

Farber E. 1994. Programmed cell death: necrosis vs. apoptosis. Mod Pathol 7:605–609.

Formigli L, Papucci L, Tani A, Schiamone N, Tempestini A, Orlandini GE, Capaccioli S, Zecchi Orlandini S.2000. Aponecrosis: morphological and biochemical exploration of a syncretic process of cell death sharing apoptosis and necrosis. J Cell Physiol 182:41–49.

Giocanti N, Hennequin C, Balosso J, Mahler M, Favaudon V. 1993. DNA repair and cell cycle interactions in radiation sensitization by the topoisomerase II poison etoposide. Cancer Res 53:2105–2111.

Govoni M, Neri S, Labella T, Sylvester JE, Novello F, Pession A. 1995. Topoisomerase-II-mediated DNA cleavage within the human ribosomal genes. Biochem Biophys Res Commun 213:282–288.

Grossmann J. 2002. Molecular mechanisms of detachment-induced apoptosis: Anoikis. Apoptosis 7:247–260.

Hengartner MO. 2000. The biochemistry of apoptosis. Nature 407: 770–775.

Kanduc D, Mittelman A, Serpico R, Sinigaglia E, Sinha AA, Natale C, Santacroce R, Di Corcia MG, Lucchese A, Dini L, Pani P, Snatacroce S, Somone S, Bucci R, Farber E. 2002. Cell death: apoptosis versus necrosis. Int J Oncol 21:165–170.

Kerr JFR, Wyllie AH, Currie AR. 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26:239–257.

Lee DH, O'Connor TR, Pfeifer GP. 2002. Oxidative DNA damage induced by copper and hydrogen peroxide promotes CG→ TT tandem mutations in nucleotide excision repair-deficient cells. Nucleic Acids Res 30:3566–3573.

Luchetti F, Burattini S, Ferri P, Papa S, Falcieri E. 2002. Actin involvement in apoptotic chromatin changes of hemopoietic cells undergoing hyperthermia. Apoptosis 7:143–152.

Majno G, Joris I. 1995. Apoptosis, oncosis and necrosis: an overview of cell death. Am J Pathol 146:3–15.

Martelli AM, Bortul R, Fackelmayer F, Tazzari PL, Bareggi R, Narducci P, Zweyer M. 1999a. Biochemical and morphological characterization of the nuclear matrix from apoptotic HL60 cells. J Cell Biochem 72:35–46.

Martelli AM, Bortul R, Bareggi R, Grill V, Narducci P, Zweyer M. 1999b. Biochemical and morphological changes in the nuclear matrix prepared from apoptotic HL-60 cells. Effect of different stabilizing procedures. J Cell Biochem 74:99–110.

Russo A, Palumbo M, Scifo C, Cardile V, Barcellona, ML, Renis M. 2001. Ethanol-inuced oxidative stress in rat astrocytes: role of HSP70. Cell Biol Toxicol 17:153–168.

Shah GM, Shah RG, Poirier GG. 1996. Different cleavage patterns for poly(ADP-ribose) polymerase during necrosis and apoptosis in HL-60 cells. Biochem Biophys Res Commun 229:838–844.

Sugimoto K, Yamada K, Egashira M, Yazaki Y, Hirai H, Kikuchi A, Oshimi K. 1998. Temporal and spatial distribution of topoisomerase II alters during proliferation, differentiation, and apoptosis in HL-60 cells. Blood 91:1407–1417.

Thielmann HW, Popanda O. 1998. Doxorubicin and γ rays increase the level of DNA topoisomerase IIαin nuclei of normal and xeroderma pigmentosum fibroblasts. J Cancer Res Clin Oncol 124:355–366.

Wyllie AH, Kerr JFR, Currie AR. 1980. Cell death: the significance of apoptosis. Int Rev Cytol 68:251–306.

Zini N, Martelli AM, Sbatelli P, Santi S, Negri C, Astaldi Ricotti GC, Maraldi NM. 1992. The 180-kDa isoform of topoisomerase II is localized in the nucleolus and belongs to the structural elements of the nucleolar remnant. Exp Cell Res 200:460–466.

Zini N, Santi S, Ognibene A, Bavelloni A, Neri LM, Valmori A, Mariani E, Negri C, Astaldi-Ricotti GC, Maraldi NM. 1994. Discrete localization of different DNA topoisomerases in HeLa and K562 nuclei and subnuclear fractions. Exp Cell Res 210:336–348.