INDUCTION OF APOPTOTIC DNA FRAGMENTATION AND CELL DEATH IN U937 HUMAN CELLS BY ULTRAVIOLET B IRRADIATION

Eduardo FERNANDEZ-SEGURA[1], Francisco J. CAÑIZARES[1], Maria A. CUBERO[1], Asunción OLMO[2], Francisco REVELLES[1] and Antonio CAMPOS[1]
[1]*Department of Cell Biology,* [2]*Department of Pathology, School of Medicine, University of Granada, E-18071 Granada, Spain*

Apotosis is a mode of cell death that is involved in the control of cell populations during development, immune responses, and normal tissue homeotasis. Apoptosis is characterized by a series of morphological changes including cell shrinkage, chromatin condensation, and apoptotic body formation. A biochemical hallmark of apoptosis is extensive degradation of DNA into nucleosomal units (Compton, 1992). This degradation is originated by the activation of an endogenous nuclear endonuclease that cleaves chromatin at internucleosomal regions. In contrast, necrosis is a mode of cell death characterized by increased membrane permeability and random degradation of DNA by lysosomal enzymes. Previous studies have demonstrated that apoptosis may be induced by a wide range of non-physiological stimuli including anti-cancer drugs, ionizing radiation, and hyperthermia (Barry at al. 1990). Recently, Martin and Cotter (1991) demonstrated that ultraviolet B (UV) irradiation induces extensive and rapid apoptosis in HL-60 cells. Actually, UV irradiation is used as a trigger of apoptosis to study the intracellular proteinases involved in the DNA fragmentation (Wright et al. 1994). In this report, we describe the temporal sequence of changes in nuclear morphology, cell shape, and DNA fragmentation during the process of apoptosis induced by UV irradiation in U937 cells.

The U937 histiocytic lymphoma cell line was kindly by Dr. López-Rivas (Instituto de Parasitologia y Biomedicina, CSIC, Granada). Cells were maintained in antibiotic-free RPMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine, and 25 mM Hepes buffer in a humid atmosphere containing 5% CO_2. Apoptosis was induced in U937 by exposure to UV B irradiation as described Martin and Cotter (1991). Briefly, cells (0.5-1 x 10^6 cel/ml) were seeded in 25 cm^2 tissue culture flasks and exposed from below to a 302-nm UV transilluminator for 10 min at room temperature. Cells were then return to 37° C and determined for apoptosis at appropriate time periods. Cell viability was assayed by trypan blue exclusion. Cells resuspended in culture medium were stained by addition an equal volume of 0.4% trypan blue (Sigma, St.Louis, Mo) in PBS and counted using a Neubauer haemocytometer.

The fluorescent dye Hoechst 33342 (bisbenzimide, Sigma, St. Louis, Mo) was used to visualized the morphological features of nuclei in U937 cells. UV light-treated and control cells were fixed with 4% paraformolaldehyde in PBS, pH 7.2, treated with Hoechst 33342 (5µg /ml) in PBS for min at 37°C, and washed with PBS, pH 7.2. Cells were dropped onto glass slides, mounted with PBS/glycerol, and visualized in a Leitz Laborlux 12 epifluorescence microscope.

For scanning electron microscopy (SEM) studies, UV light-treated and control cells were removed from culture medium, washed with 0.1 M buffer phosphate, pH 7.2, and fixed with 2.5% glutaraldehyde in 0.1 M buffer phosphate, pH 7.2, at room temperature. Cells were fixed overnight at 4°C, then washed (3 times) with 0.1 M buffer phosphate, pH 7.2, and dropped onto poly-L-lysine precoated coverslips. The cells were dehydrated, critical-point dried with CO_2, and gold-sputter coated. The samples were visualized in a Philips XL30 (Philips, Eindhoven, The Netherlands) scanning electron microscope.

The internucleosomal DNA fragmentation was assayed by a modified method of Nieto and López-Rivas (1989).Biefly, UV light-treated and control cells (2 x 10^6 cel/ml) were washed (2 times) with isotonic PBS, pH 7.4, and disrupted by a lysis buffer containing 20 mM Tris-HCl, pH 7.3, 2 mM EDTA, and 0.4% (v/v) Triton X-100 for 15 min at 4°C. Cellular lysates were then centrifuged at 15000 rpm for 15 min to separate the low molecular weight DNA from the intact chromatin. Fragmented DNA in the supernatant was treated with RNase A (20 µg/ml) and proteinase K (100 µg/ml) for 3 h at 50°C. After incubation, fragmented DNA was extracted with phenol:chloroform:isoamyl alcohol and precipitated with ethanol. Electrophoresis of fragmented DNA was carried out on 1% agarose gels in TAE buffer, pH 8.0, and stained with ethidium bromide.

Exposure of U937 cells to UV light for 10 min induced time-dependent morphological changes in nuclei and cell shape. To evaluate the nuclear morphology changes, U937 cells were stained with the fluorescent dye Hoechst 33342 (Fig. 1). Control cells showed uncondensed chromatin with low blue fluorescence. After 60 min postirradiation, U937 cells showed clumps of condensed chromatin accumulated in the nuclear periphery with high blue fluorescence. After 90 min postirradiation, U937 cells showed fragmented chromatin. These changes were accompanied by a time-dependent decrease in cell size as determined by flow cytometry (not shown). Figure 1 also shows the cell viability of U937 cells after UV irradiation. Our results demonstrate that U937 cells were >95% viable as determined using trypan blue exclusion.

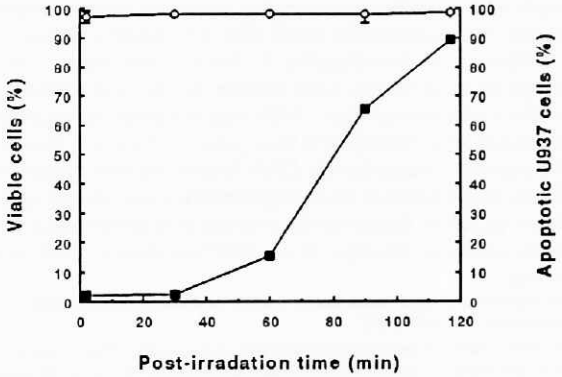

Fig. 1. Time-dependent effects of UV irradiation in U937 cells. Cells were exposed to UV irradiation for 10 min at room temperature and then incubated for 0, 30, 60, 90 and 120 min at 37°C. Cell viability was assayed by trypan blue exclusion (o). U937 apoptotic cells were determined with Hoechst 33342 (■). Results are means ± SD of three separated experiments.

To correlate the changes in nuclear morphology after UV irradiation with changes in cell shape, we used SEM. Figure 2A is a representative scanning electron micrograph of U937 control cells. This cell type displays a round-smooth surface morphology with microvilli. SEM morphological examination of U937 cells after

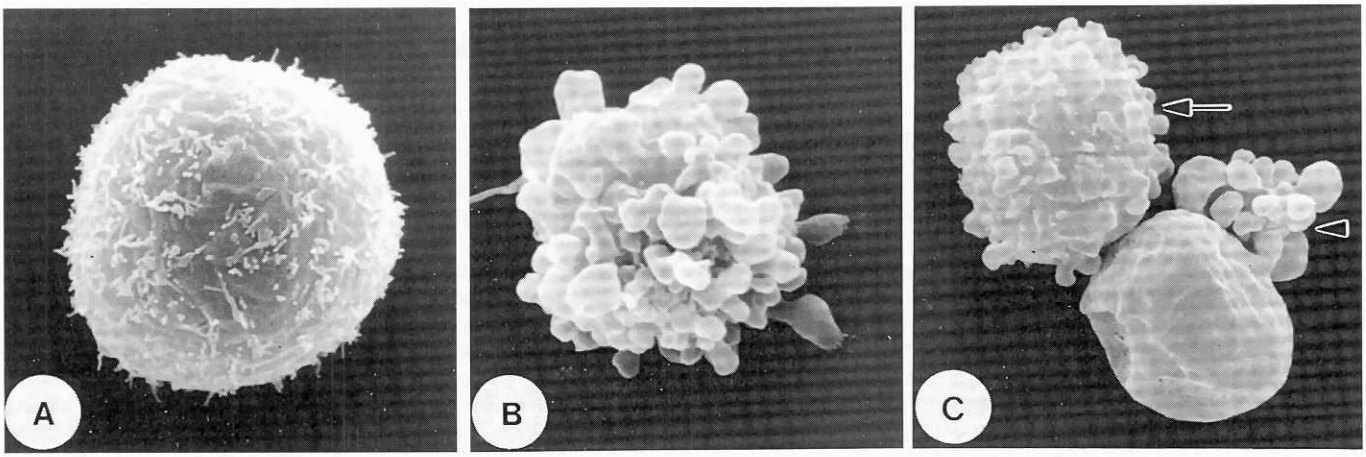

Fig. 3. Scanning electron micrographs of changes in cell shape in U937 cells. Control (A) and UV light-treated (B,C) U937 cells were fixed with 2.5% glutaraldehyde, dropped onto coverslips, dehydrated, critical point dried and gold-sputter coated. (A)) Control cells showed a microvillous cell surface morphology; (B) U937 cells with membrane blebs; (C)U937cells showing membrane blebs (arrow) and membrane-bounded apoptotic bodies (arrowhead).

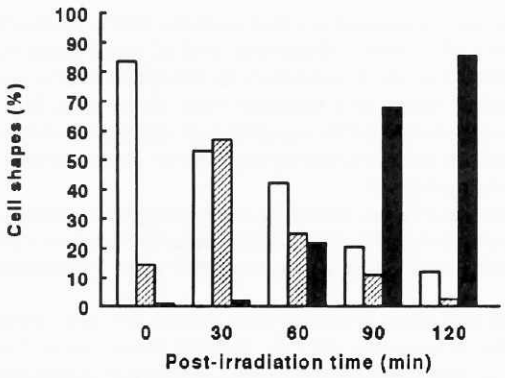

Fig. 2. Time course of changes in cell shape in U937 cells after irradiation with UV light for 10 min at room temperature. Control cells (*open bars*); U937 cells with blebs (*filled bars*); U937 cells with membrane-bounded apoptotic bodies *(closed bars*).

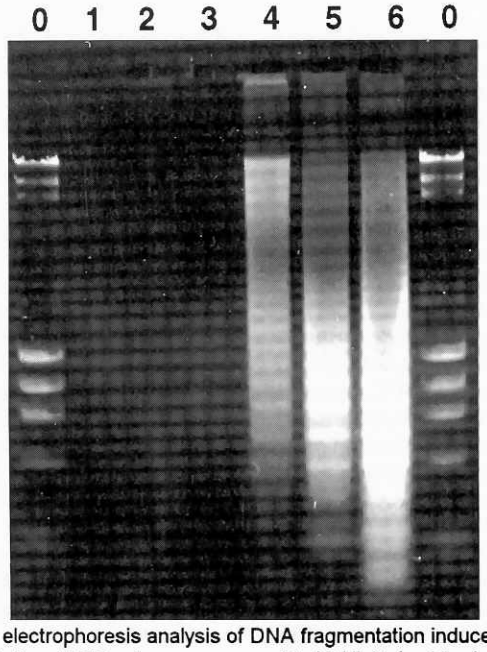

Fig. 4. Gel electrophoresis analysis of DNA fragmentation induced by UV light irradiation. U937 cells were exposed to UV light for 10 min at room temperature and then incubated for 0, 30, 60, 90, and 120 min at 37°C. Lane 0, molecular weight markers; Lane 1, control cells; Lane 2, 0 min postirradiation; Lane 3, 30 min postirradiation; Lane 4, 60 min postirradiation; Lane 5, 90 min postirradiation; Lane 6, 120 min postirradiation.

exposure to UV light allowed us to identify two cell types: cells with extensive membrane blebs (Fig. 3B), and cells with membrane-bounded apoptotic bodies (Fig. 3C). Figure 3 illustrates the time-course of changes in cell shape in U937 cells after UV irradiation. U937 cells with membrane-bounded apoptotic bodies increased after 60 min, and reached its peak after 2 h postirradiation.

In addition, we investigated if these changes in nuclear morphology and cell shape were accompanied by the cleavage of DNA at internucleosomal sites. DNA was analyzed by agarose gel electrophoresis after appropriate time periods. Figure 4 shows that UV light induces characteristic DNA fragmentation of apoptosis. After 60 min postirradiation, DNA fragmentation was already visualized in U937 cells. No DNA fragmentation was detected in U937 control cells. Taken together, these results indicate that ultraviolet B irradiation-induced apoptosis in U937 cells. In addition, these findings show the relation between changes in nuclear morphology, cell shape, and DNA fragmentation.

References

Barry, M.A., Behnke, C.A. and Eastman, A. (1990) Activation of programmed cell death (apoptosis) by cisplatin, other anticancer drugs, toxins and hyperthermia. Biochem. Pharmacol. 40:2353-2362.

Compton, M.M. (1992). A biochemical hallmark of apoptosis: internucleosomal degradation of genome. cancer Metastasis Rev. 11:105-119.

Martin, S.J. and Cotter, T.G. (1991) Ultraviolet B irradiation of human leukaemia HL-60 cells *in vitro* induces apoptosis. Int. J. Radiat. Biol. 59:1001-1016.

Nieto, M.A. and Lopez-Rivas, A. (1989). IL-2 protects T lymphocytes from glucocorticoid-induced DNA fragmentation and cell death. J. Immunol. 143:4166-4170.

Wright, S.C., Wei, Q.S., Zhong, J., Zheng, H., Kinder, D.H. and Larrick, J.W. (1994). Purification of a 24-kD protease from apoptotic tumor cells that activates DNA fragmentation. J. Exp. Med. 180:2113-2123.