Ultraviolet radiation (UV) induces reorganization of actin cytoskeleton in CHOAA8 cells

D. GRZANKA[1], J. DOMANIEWSKI[1], A. GRZANKA[2], A. ZURYN[2]

[1]Department of Clinical Pathomorphology, e-mail: agrzanka@cm.umk.pl, and [2]Department of Histology and Embryology, Nicolaus Copernicus University, Collegium Medicum, 85-094 Bydgoszcz, Poland

Received December 7, 2005

Effect of UV radiation on actin cytoskeleton was studied in CHOAA8 cells by fluorescence and electron microscopy. UV irradiated cells showed impaired adherence, disruption of the actin filaments and stronger F-actin labeling in the center of the cell. Attached cells, especially enlarged ones showed rather weak labeling of stress fibers and bundles of F-actin in the cytoplasm, but some cells with intensive labeling of these structures were also observed. Detached cells were rounded, showed strong F-actin labeling and often had buds. At the ultrastructural level UV-irradiated cells showed segmented nuclei, bodies resembling micronuclei, dilatation of endoplasmic reticulum, swollen and disturb mitochondria. Immunogold labeling of actin at the ultrastructural level was observed in non-radiated and UV irradiated cells. Actin labeling was seen in nuclei and cytoplasm. In nuclei gold particles were localized in the area of condense chromatin. Labeling for actin was not found after control incubation. Our observations show that UV radiation promotes changes in the distribution of actin in CHOAA8 cells. The results also suggest that not only reorganization of actin but changes in organelles are involved in the process of apoptosis initiated by UV radiation.

Key words: CHOAA8 cells, actin, UV radiation, apoptosis, immunogold technique, fluorescence method

Biological effects of ultraviolet radiation (UV) arise from DNA damage. UV initiates in cells mechanism that involve activation of DNA damage response pathway, cell cycle arrest and apoptosis [1]. Little is known what is the of effect UV radiation on the cytoskeleton. MALORNI et al in their work showed that the reactive oxygen species mediated oxidative modification of membrane lipids and cytoskeletal proteins as a consequence of UV radiation [2]. There are other reports on the direct effects of UVA or UVB radiation on cytoskeletal proteins in cells *in vitro* [3–7]. It has also been shown that UV radiation mediated changes in the structure of cytoskeletal proteins which are linked to the defect in polymerization and network organization [6, 7]. VESELSKA et al reported changes in the cytoskeletal proteins after UV radiation which were manifested as atypical arrangements of actin fibres and their asymmetric distribution in relation to nucleus. Her work showed that microtubules were more sensitive to UV radiation than microfilaments [8]. There are reports on the effect of radiation on DNA in Chinese hamster ovary cells (CHO) [9–11] but not any on reorganization of actin. We have previously described the changes in the organization of actin, especially in nuclei in relationship to chromatin reorganization during process of apoptosis [12–14]. Studies on apoptosis in relation to actin cytoskeleton have also been attracting attention of other researches [15–20]. In the future the link between actin and apoptosis may offer new opportunities for therapeutic intervention, such as in the treatment of cancer or other disorders. Understanding not only the molecular control of apoptosis but also signaling to the actin cytoskeleton which is involved in apoptosis will undoubtedly yield a new generation of drug targets. However, there are many key questions still to be answered.

Material and methods

Chinese hamster ovary cells, CHO AA8 were kindly provided by Prof. M. Zdzienicka from Leiden University Medical Center. Cells were diluted to $5x10^5$ cells/ml and grown in RPMI 1640 medium supplemented with 15% fetal calf serum and 125 µl gentamycin in a humidified atmosphere in 5% CO_2 at 37 °C. The cells were cultured for 66 hours, then were

UVC irradiated for 2 min at a fluency of 100 J/m^2 using Philips G30T8 lamp and culture was continued for 6 more hours. Control cells were treated identically but not irradiated. Cell viability was assessed by the trypan blue dye exclusion method. F-actin filaments in cells were labeled with BODIPY FL phallacidin, (Molecular Probes). Cells for fluorescent microscopical analysis were fixed with 4% paraformaldehyde. After fixation cells were incubated in 2 μM BODIPY FL in calcium and magnesium free phosphate-buffered saline (PBS-A) containing 20% methanol for 20 min at a room temperature in the dark, rinsed three times in PBS-A and mounted in gelvatol [21]. The organization of F-actin filaments was examined with an Eclipse E600 microscope equipped with Y-FL epifluorescence equipment (Nikon, Tokyo, Japan) and with confocal microscope (inverted microscope, Nikon, Eclipse TE 300). For conventional electron microscopy cells were fixed in 3.6% glutaraldehyde in phosphate buffer, postfixed in OsO_4 in the same buffer, dehydrated in alcohol series and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate. To visualize actin at the ultrastructural level, a postembedding streptavidin-gold method was used. Cells were fixed in 4% paraformaldehyde in PBS for 1 h at 4 °C, washed overnight in PBS at 4 °C, dehydrated in series of ethanol and embedded in LR White. Sections were collected on nickel grids (Sigma). Grids were floated on a drop of nonimmune rabbit serum (Dako, Glostrup, Denmark) for 20 min and then transferred onto drops of monoclonal anti-actin antibody (AC-40; Sigma) diluted 1:100. After incubation with primary antibody for 30 min, thin sections on grids were rinsed in PBS and incubated with biotinylated rabbit anti-mouse antibody (Dako) diluted 1:100. Grids were then rinsed in PBS and transferred onto drops of solution containing 10 nm gold particles conjugated to streptavidin (Sigma) 1:20 dilution, and incubated for 30 min. After this final incubation grids were washed in PBS and dried. All incubation steps were performed at room temperature. Control specimens were incubated with nonimmune antiserum (normal mouse serum, Dako). The preparations were examined using a transmission electron microscope JEM 100 CX (JEOL, Tokyo, Japan) at 80 kV.

Results

Actin cytoskeleton in control cells is shown in Figure 1. After UV irradiation cells showed rather impaired adherence. Cells became more rounded and there were also attached cells with marked increase in size. We have also found UV-induced disruption of the actin filaments and often increase in bright F-actin labeling in the center of the cell. Attached cells especially the big ones show rather weak stress fibers and bundles of F-actin labeling in the cytoplasm (Fig. 2). Detached cells were rounded with strong F-actin labeling. Some cells have buds labeled for F-actin (Fig. 3). Shrunk cells with nuclear fragmentations were also observed. At the ultrastructural level control cells showed normal morphology with oval or kidney-shaped single nucleus (Fig.4a). UV-irradiated cells showed segmented nuclei that appeared as isolated ones and bodies with heterochromatin resembling micronuclei (Figs. 4b, 4c). Nuclei with diffuse areas of condensed chromatin were also seen (Fig. 4d). Additional changes like dilatation of endoplasmic reticulum, swollen and disturb mitochondrium, vesiculation of cytoplasm were observed (Fig. 5a). Numerous cells have areas where affected mitochondria and bodies containing chromatin-like material were observed (Fig. 5b). The distribution of actin at the ultrastructural level using immunogold labeling was observed in both UV irradiated and control cells. Actin in UV irradiated CHOAA8 cells in the nucleus was especially local-

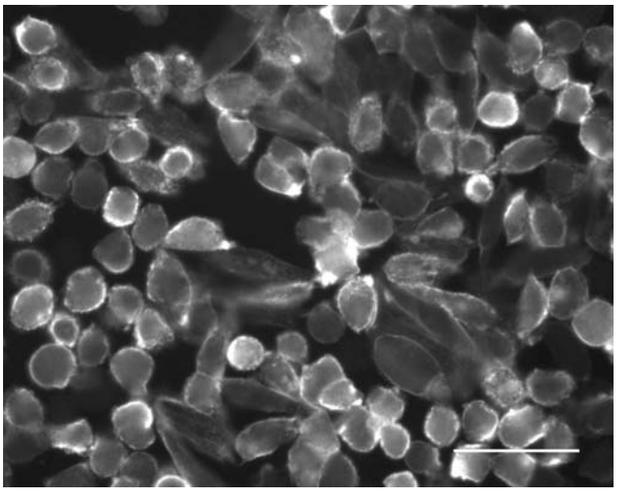

Figure 1. Control. Not UV-irradiated CHOAA8 cells. Bar, 50 μm.

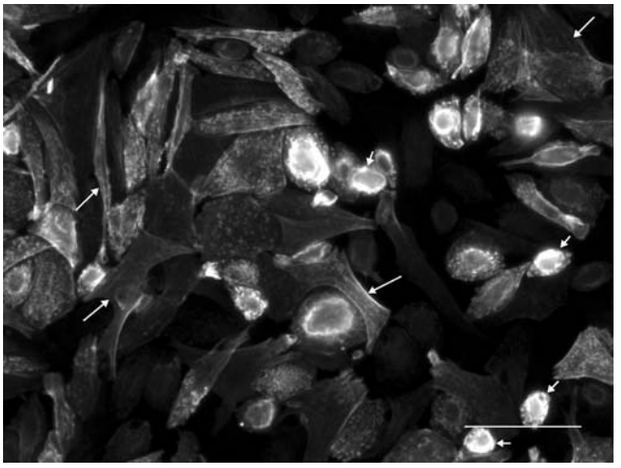

Figure 2. UV irradiated CHOAA8 cells labeled with phallacidin-Bodipy. Elongated, adherent cells (long arrows) and also rounded, detached with strong F-actin in the center are seen (short arrows). Bar, 40 μm.

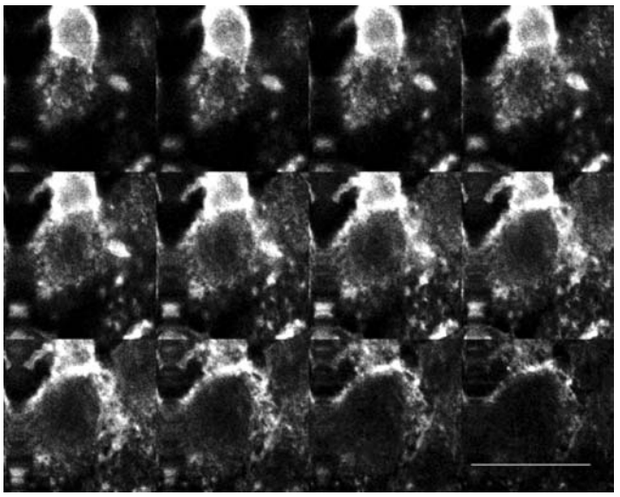

Figure 3. Z-scan series through UV treated CHOAA8 cell with phallacidin-Bodipy labeling for F-actin. Rich F-actin labeling in bud

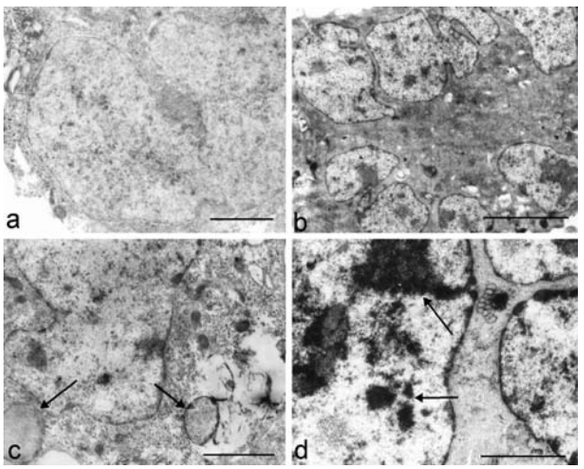

Figure 4. a) Electron micrograph of not UV-irradiated CHOAA8 cell. The kidney sharped single nucleus is seen. Bar, 2 µm; b) CHOAA8 cell irradiated with UV. Visible nucleus segmentation with total isolation of its segments. Bar, 2 µm; c) Electron micrograph of CHOAA8 cell after UV radiation. Bodies resembling micro-nuclei are seen (arrows). Bar, 1 µm; d) CHOAA8 cell section after UV induction. Visible diffuse areas of condensed chromatin in nucleus (arrows). Bar, 0.5 µm.

ized in the areas of condense chromatin (Fig. 5c). Actin labeling was not found in control cells incubated with non-immune serum (Fig. 5d).

Discussion

Here, we have demonstrated reorganization of actin cytoskeleton in CHOAA8 cells induced by ultraviolet radiation UVC. Apoptosis induced by UV is driven by two major pathways, DNA damage and death receptor activation [22]. It is known that UV induces oxidative stress reactions which

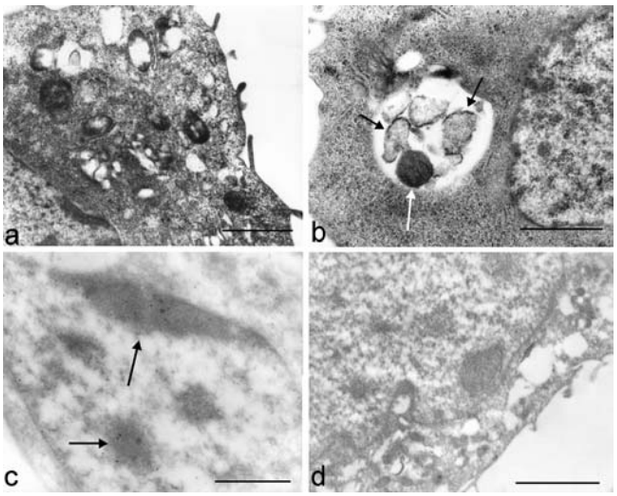

Figure 5. a) CHOAA8 cell section after UV-irradiation. Changed mitochondria, endoplasmatic reticulum as well as cytoplasmic vacuolization can be observed. Bar, 1 µm; b) Electron micrograph of CHOAA8 cell UV irradiated. Swollen mitochondrium and bodies with material similar to chromatin (black arrow) are present in the intracellular space (white arrow). Bar, 1 µm; c) Electron micrograph of CHOAA8 cell after UV radiation. Gold particles are localized in areas of condense chromatin Bar, 1 µm; d) Electron micrograph of CHOAA8 cell showing the control reaction with normal mouse serum instead of primary antibodies. Bar, 1 µm.

play a role in apoptosis [23]. It has been reported that UV irradiation induces degradation of cytoskeletal proteins in cells and leads to disturbance of cell adherence and then to apoptosis [2, 4, 7, 8, 20, 22, 24, 25]. The actin cytoskeleton may represent an additional pathway involved in the process of apoptosis and can be a promising target for development of new chemotherapies. Further investigations on involvement of actin system in apoptosis can provide a new insight into that process and may have significant clinical interest. The mechanisms by which fibroblasts undergo apoptosis are still not well understood. It is known that cells activate the apoptotic pathway to eliminate one self with damaged DNA but still is not clear how these DNA damages induce apoptosis. There are studies on CHO cells which showed that c-myc degradation induced apoptosis [26]. Other studies also showed that the c-myc proto-oncogene was required for DNA damage-induced apoptosis in G2 phase of rat embryo fibroblasts [27]. Here, we demonstrate the effect of UV irradiation on actin cytoskeleton in CHOAA8 cells which are functionally defective in p53 [28, 29]. We observed cells with characteristic features of apoptosis and there were relationship of actin reorganization with characteristic morphological features of apoptosis. Previously was showed that UVB induces cytoskeleton dependent surface blebbing and the type of apoptosis called anoikia in A431 epithelial cells [4, 30]. Kulms et al in their studies demonstrated that alterations of the cytoskeleton trigger death receptors CD95 (Fas/APO-1) and suggest that this may have influence on

UV-induced apoptosis. They concluded that it can be additional pathway involved in the complex process of UV-induced apoptosis. Their data suggest that disruption of the cytoskeleton causes apoptosis via activation of CD95 and enhances UV-induced apoptosis, possibly via aiding receptor clustering [20]. Here, we showed not only reorganization of actin but also changes in other organelles which have not received much attention so far, but they may play role in the control of cell death. We have observed dilation of endoplasmic reticulum and vacuoles in apoptotic-like cells. The accumulation of electron dense material in the intervacuolar space was also observed, what may suggest the autophagic process, such as an uptake of mitochondrium into lysosomes. NICOTERA et al in their studies observed mixed apoptotic-necrotic features including cellular swelling, blebbing, nuclear pyknosis, display of phosphatidylserine, and uptake of mitochondrium into lysosmes [31]. There are also other studies showing that other organelles such as the endoplasmic reticulum and lysosomes, may also play an essential role in the control of cell death [32–34] Our findings suggest that actin and other organelles can be involved in the process of apoptosis in CHOAA8 cells.

References

[1] GENTILE M, LATONEN L, LAIHO M. Cell cycle arrest and apoptosis provoked by UV radiation induced DNA damage are transcriptionally highly divergent responses. Nucleic Acids Res 2003; 31: 4779–4790.

[2] MALORNI W, IOSI F, MIRABELLI F, BELLOMO G. Cytoskeleton as a target in menadione-induced oxidative stress in cultured mammalian cells: alterations underlying surface bleb formation. Chem Biol Interact 1991; 80: 217–236.

[3] ZAMANSKY GB, CHOU IN. Environmental wavelengths of ultraviolet light induce cytoskeletal damage. J Invest Dermatol 1987; 89: 603 -606.

[4] MALORNI W, DONELLI G, STRAFACE E, SANTINI MT, PARADISI S et al. Both UVA and UVB induce cytosceleton dependent surface blebbing in epidermoid cells. J Photochem Photobiol B 1994; 26: 265–270.

[5] ZAMANSKY GB, NGUYEN U, CHOU IN. An immunofluorescence study of the effect of ultraviolet radiation on the organization of microfilaments, keratin intermediate filaments, and microtubules in human keratinocytes. Cell Motil Cytoskeleton 1992; 22: 296–306.

[6] ZAMANSKY GB, PERRINO BA, CHOU IN. Disruption of cytoplasmic microtubules by ultraviolet radiation. Exp Cell Res 1991; 195: 269–273.

[7] DJAVAHERI-MERGNY M, PIERAGGI MT, MAZIERE C, SANTUS R, LAGERON A et al. Early alterations of actin in cultured human keratinocytes and fibroblast exposed to long-wavelenght radiations. Possible involvement in the UVA-induced perturbations of endocytotic processes. Photochem Photobiol 1994; 59: 48–52.

[8] VESELSKA R, JANISCH R. The effect of UV irradiation on changes in cytoskeleton and viability of mouse fibroblasts L929 cell line. Scripta Medica (Brno) 2000; 73: 393–408.

[9] TZANG BS, CHIANG YJ, LAN HC, LIAO CB, LIU YC. Tuning up or down the UV-induced apoptosis in Chinese hamster ovary cells with cell cycle inhibitors . Photochem Photobiol 2002; 75: 662–667.

[10] HENTOSH P, COLLINS AR, CORRELL L, FORNACE AJ JR, GIACCIA A ET AL. Genetic and biochemical characterization of CHO-UV-1 mutant defective in postreplication recovery of DNA. Cancer Res 1990; 50: 2356–2362.

[11] THOMPSON LH, MITCHELL DL, REGAN JD, BOUFFLER SD, STEWART SA et al. CHO mutant UV61 removes (6-4) photoproducts but not cyclobutane dimers. Mutagenesis 1989; 4: 140–146.

[12] GRZANKA A. Actin distribution patterns in HL-60 leukemia cells treated with etoposide. Acta Histochem 2001; 103: 453–464.

[13] GRZANKA A, GRZANKA D, ORLIKOWSKA M. Cytoskeletal reorganization during process of apoptosis induced by cytostatic drugs in K-562 and HL-60 leukemia cell lines. Biochem Pharmacol 2003; 66: 1611–1617.

[14] GRZANKA D, DOMANIEWSKI J, GRZANKA A. Actin reorganization in Chinese hamster ovary (CHOAA8) cells induced by doxorubicin. Neoplasma 2005; 1: 46–51.

[15] COTTER TG, LENNON SV, GLYNN JM, GREEN DR. Microfilament-disrupting agents prevent the formation of apoptotic bodies in tumor cells undergoing apoptosis. Cancer Res 1992; 52: 997–1005.

[16] LEVEE MG, DABROWSKA MI, LELLI JL, HINSHAW DB. Actin polymerization and depolymerization during apoptosis in HL-60 cells. Am J Physiol 1996; 271: C1981–C1992.

[17] LUCHETTI F, BURATTINI S, FERRI P, PAPA S, FALCIERI E. Actin involvement in apoptotic chromatin changes of hemopoietic cells undergoing hyperthermia. Apoptosis 2002; 7: 143–152.

[18] OSHIMA RG. Apoptosis and keratin intermediate filaments. Cell Death Differ 2002; 9: 486–492.

[19] RODOLFO C, PIACENTINI M. Does cytoskeleton 'Akt' in apoptosis? Cell Death Differ 2002; 9: 477–478.

[20] KULMS D, DUSSMANN H, POPPELMANN B, STANDER S, SCHWARZ A, SCHWARZ T. Apoptosis induced by disruption of actin cytoskeleton is mediated via activation of CD95 (Fas/APO-1). Cell Death Differ 2002; 9: 598–608.

[21] BELL PB, SAFIEJKO-MROCZKA B. Visualization of macromolecular structures by fluorescence and scanning electron microscopy. Scan Microsc 1995; 9 (3): 943–960.

[22] KULMS D, POPPELMANN B, YAROSH D, LUGER TA, KRUTMANN J et al. Nuclear and cell membrane effects contribute independently to the induction of apoptosis in human cells exposed to UVB radiation. Proc Natl Acad Sci USA 1999; 96: 7974–7979.

[23] STRAFACE E, NATALINI B, MONTI D, FRANCESCHI C, SCHETTINI C et al. C3-fullero-tris-methanodicarboxylic acid protects epithelial cells free radiation-induced anoikia by influencing cell adhesion ability. FEBS Lett 1999; 454: 335–340.

[24] WEINREB O, DOVRAT A, DUNIA I, BENEDETTI EL, BLOEMENDAL H. UV-A-related alterations of young and adult lens water-insoluble alpha-crystallin, plasma membranous and cytoskeletal proteins. Eur J Biochem 2001; 268: 536–543.

[25] MALORNI W, STRAFACE E, DONELLI G, GIACOMONI PU.

UV-induced cytoskeletal damage, surface blebbing and apoptosis are hindered by -tocopherol in cultured human keratinocytes. Eur J Dermatol 1996; 6: 414–420.

[26] JIANG MR, LI YC, YANG Y, WU JR. c-Myc degradation induced by DNA damage results in apoptosis of CHO cells. Oncogene 2003; 22: 3252–3259.

[27] ADACHI S, OBAYA AJ, HAN Z, RAMOS-DESIMONE N, WYCHE JH et al. Mol Cell Biol 2001; 21: 4929–4937.

[28] LARNER JM, LEE H, HAMLIN JL. Radiation effects on DNA synthesis in a defined chromosomal replicon. Mol Cell Biol 1994; 14: 1901–1908.

[29] LEE H, LARNER JM, HAMLIN JL. Cloning and characterization of Chinese hamster p53 cDNA. Gene 1997; 184: 177–183.

[30] FIORENTINI C, MATARRESE P, STRAFACE E, FALZANO L, FABBRI A et al. Rho-dependent cell spreading activated by E.coli cytotoxic necrotizing factor 1 hinders apoptosis in epithelial cells. Cell Death Differ 1998; 5: 921–929.

[31] NICOTERA P, LEIST M, MANZO L. Neuronal cell death: a demise with different shapes. Trends Pharmacol Sci 1999; 20: 46–51.

[32] MATTSON MP. Apoptosis in neurodegenerative disorders. Nat Rev Mol Cell Biol 2000; 1: 120–129.

[33] LEIST M, JAATTELA M. Triggering of apoptosis by cathepsins. Cell Death Differ 2001; 8: 324–326.

[34] FERRI KR, KROEMER G. Organelle-specific initiation of cell death pathways. Nat Cell Biol 2001; 3: E255–E263.