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X-IRRADIATION-INDUCED DISORGANIZATION OF CYTOSKELETAL FILAMENTS AND CELL CONTACTS IN HT29 CELLS

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Abstract

Organization of cytoskeleton and cell contacts were studied by immunochemistry and electron microscopy in confluent HT29 cultured cells following exposure to 0.5 and 1.0 Gy doses of X-ray. Microtubules were resistant to irradiation, whereas, the actin and intermediate filaments disrupted rapidly following the treatment and their components appeared as clumps of actin and cytokeratin aggregates in the cytoplasm as demonstrated by immunochemistry. Loss of cell contacts and decrease in the number of desmosomes was also characteristic of irradiated cells. Electron microscopy revealed intact desmosomes in control cells and abnormal desmosomes in the irradiated samples characterized by the absence of tonofilaments. The perinuclear filament network and cortical filaments were well detectable by electron microscopy. Under the effect of irradiation, the perinuclear filaments almost disappeared and, at the same time, small bundles of filaments were formed irregularly in the cytoplasm associated with amorphous material.

Key Words: X-irradiation, cytoskeleton, microtubules, actin, cytokeratin, vinculin, vimentin, intermediate filaments, immunochemistry, electron microscopy, HT-29 cells.

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Introduction

All eukaryotic cells contain three major classes of cytoskeletal elements, the actin microfilaments, the microtubules and the intermediate filaments (Coulombe, 1993; Stewart, 1993; Cramer *et al.*, 1994). The cytoskeleton determines the general shape of the cell, regulates the movements of cells and subcellular structures, maintains different kinds of cell contacts, and takes part in cell to cell communication (Barrit, 1992; Coulombe, 1993; Reisler, 1993; Stewart, 1993; Cramer *et al.*, 1994; Hirokava, 1994; Hitt and Luna, 1994; Mays *et al.*, 1994; Amos, 1995, Wallee and Okamoto, 1995).

The actin based filaments and the microtubular network are continuously changing structures, the assembly and disassembly of which is regulated by associated proteins and by numerous of intracellular and extracellular factors (Reisler, 1993; Cramer *et al.*, 1994; Hirakova, 1994; Hitt and Luna, 1994; Amos, 1995; Cleveland, 1995; Small, 1995). The intermediate filaments (IF) are less dynamic structures. Nevertheless, their assembly and disassembly during the different steps of cell cycle is well documented. This type of filament consists of several classes including cytokeratins in epithelial cells, desmin in muscles, neurofilaments in neurons, glial fibrillary acidic protein in glial cells, and vimentin in cells with mesenchymal origin. The nuclear lamins are also related to IFs (Coulombe, 1993; Stewart, 1993, Heins and Aebi, 1994; Mays *et al.*, 1994). Elements of cytoskeleton can be associated with the cell membrane and participate in formation and/or stabilization of various cell-cell and cell-matrix contacts (Ungar *et al.*, 1986; Garrod, 1993; Hitt and Luna, 1994; Mays *et al.*, 1994). This binding between the cytoskeleton and membrane elements is usually indirect and is accomplished with the help of linker proteins including vinculin, talin and others (Ungar *et al.*, 1986; Garrod, 1993; Hitt and Luna, 1994; Mays *et al.*, 1994).

Although the chemical composition and the structural organization of the three groups of cytoskeletal elements are regulated separately in the cells, they form a consistent, harmonizing system (Green *et al.*, 1987;

Shiba *et al.*, 1987; Rahilly and Fleming, 1992; Trevor *et al.*, 1995). The assembly and disassembly of the whole structure are closely related to the phosphorylation and dephosphorylation of particular cytoskeletal proteins and in the organization of the whole system as well (Shiba *et al.*, 1987; Grant and Aunis, 1990; Colombe, 1993; Reisler, 1993; Heins and Aebi, 1994; Hirokava, 1994; Small, 1995).

Data available in the literature suggest that one of the biological effects of the irradiation is its direct or indirect influence on the cytoskeletal system. Changes in the general shape of the cell (Yau, 1981; Somosy *et al.*, 1987), modifications of cell contacts and retraction of cells (Somosy *et al.*, 1987; Kantak *et al.*, 1993), damage of the spindle apparatus (Rubin *et al.*, 1988; Cornforth and Goodwin, 1991; Raicu *et al.*, 1993), modulation of the expression of a series of cytoskeletal proteins (Anniko *et al.*, 1989; Fischer *et al.*, 1989; Nedzvetskii *et al.*, 1990; Woloschack *et al.*, 1990; Woloschack and Chang-Liu, 1991; Kasper *et al.*, 1993a, b), F-actin reorganization (Friedman *et al.*, 1986; Kantak *et al.*, 1993), and increased vascular permeability (Evans *et al.*, 1986; Law and Ahier, 1989; Somosy *et al.*, 1993) have been observed after ionizing irradiation in cells studied *in vivo* and *in vitro*.

In the present study, the X-ray induced morphological changes in the organization of the cytoskeletal system have been investigated by transmission electron microscopy (TEM) and the changes of the location of some particular proteins (cytoskeletal actin, beta-tubulin, cytokeratin, vinculin, vimentin, and desmosomal protein) have been followed by immunohistochemical methods in cultured human colon carcinoma (HT29) cells. As shown from the data of cell survival assay (Lawrence *et al.*, 1994), irradiation did not cause cell death in the used dose range (0.5-1 Gy)

Materials and Methods

Cells

The human adenocarcinoma-derived cell line HT29, originally established by Fogh and Trempe (1975), was obtained from Public Health Laboratory Service (Salisbury, U.K.). The cells were plated on glass coverslips and or Thermanox® plates (Nunc, Kastrup, Denmark) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal calf serum (FCS), antibiotics at 37°C in a 5% CO_2 moist atmosphere.

X-irradiation

X-irradiation was performed with 0.5 and 1.0 Gy doses in a THX-250 machine at room temperature, in culture medium at the following conditions: 200 kV; half value layer: 1.0 mm Cu; source-surface distance: 90 cm; and dose rate: 0.317 Gy water.min^{-1}. Cell cultures were fixed for light- or electron microscopical investigations 30 minutes, and 1, 3 and 24 hours after irradiation.

Immunohistochemistry

The immunohistochemical preparation were made by a conventional method. The HT29 cells, grown on glass coverslips in standard medium, were fixed and permeabilized in methanol at -20°C for at least 2 hours. After washing in phosphate buffered saline (PBS, pH 7.4), the unspecific binding sites were bound in 2% bovine serum albumin (BSA, fraction VI, Sigma Chem. Co., St. Louis, MO, USA) for 60 minutes. The monoclonal antibodies, developed in mice, were obtained from Sigma. Anti-tubulin (Clone no. DM 1A), anti-vinculin (Clone no. VIN-11-5), anti-vimentin (Clone no. hVIN-1), anti-cytokeratin (Clone no. CK5) and anti-desmosomal protein (Clone no. ZK-31) were applied on the coverslips in 1:50 dilution bound in PBS containing 0.2% BSA, while the anti-actin (BL/ICC, developed in rabbit, from Amersham, Braunschweig, Germany) was used at 1:100 dilution for 60 minutes at room temperature, or overnight at 4°C. After washing with PBS, the cells were incubated in the presence of FITC (fluorescein isothiocyanate)-labelled anti-mouse antibody developed in rabbit (Sigma) or rhodamine labeled anti-rabbit

Figures 1-6 (on the facing page 765). Indirect immunofluorescence of cultured HT29 cells. The nuclei were stained with ethydiumbromide in Figures 1, 2 and 5. All figures are at same magnification; photo width in Figure 1a = 48 μm.

Figure 1. Anti-tubulin staining of the microtubules. Control (a), X-irradiated with 0.5 Gy, after 24 hours (b); with 1 Gy: after 1 hour (c) and after 24 hours (d).

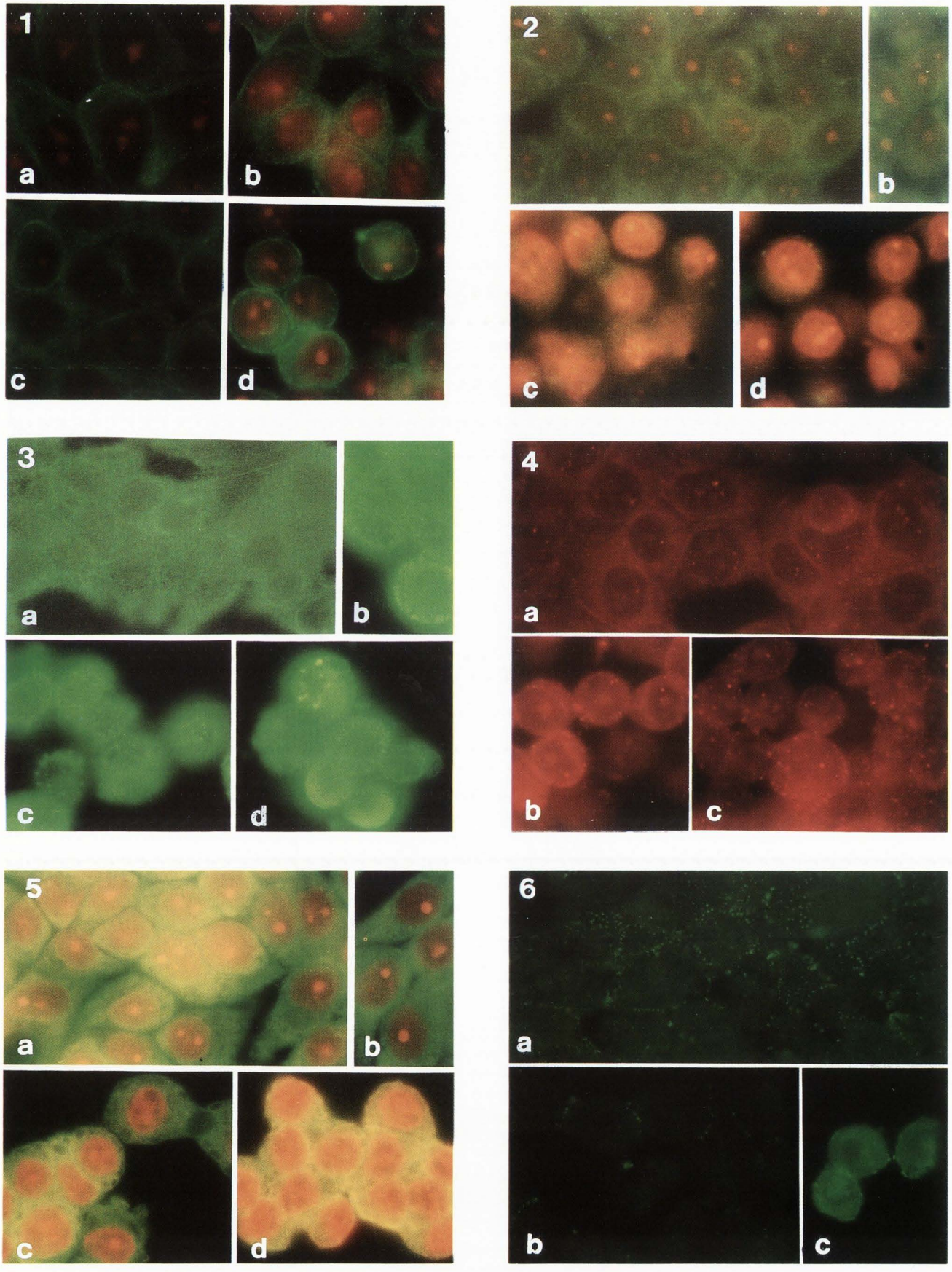
Figure 2. Anti-vimentin staining of vimentin filaments. Control (a); X-irradiated with 0.5 Gy, after 1 hour (b); with 1 Gy: after 1 hour (c) and after 24 hours (d).

Figure 3. Anti-cytokeratin staining of cytokeratins. Control (a); X-irradiated with 0.5 Gy, after 1 hour (b); with 1 Gy: after 1 hour (c) and after 24 hours (d).

Figure 4. Anti-actin staining of actin. Control (a); X-irradiated with 0.5 Gy, after 24 hours (b); with 1 Gy, after 24 hours (c).

Figure 5. Anti-vinculin staining of vinculin. Control (a); X-irradiated with 0.5 Gy, after 1 hour (b); with 1 Gy: after 1 hour (c) and after 24 hours (d).

Figure 6. Anti-desmosomal protein staining of desmosomes. Control (a); X-irradiated with 0.5 Gy, after 1 hour (b); with 1 Gy, after 24 hours (c).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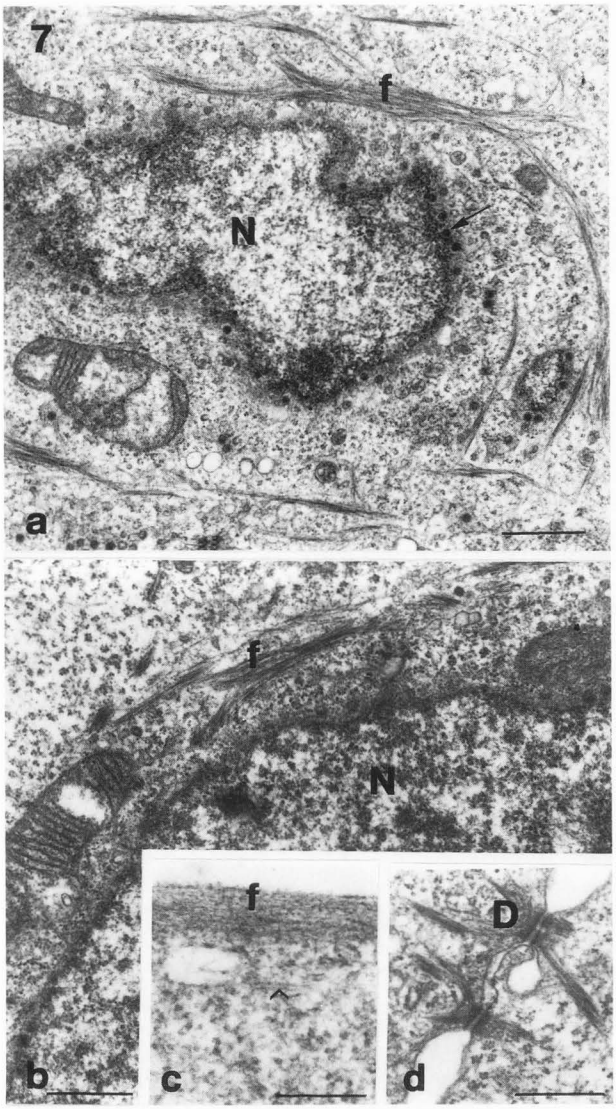

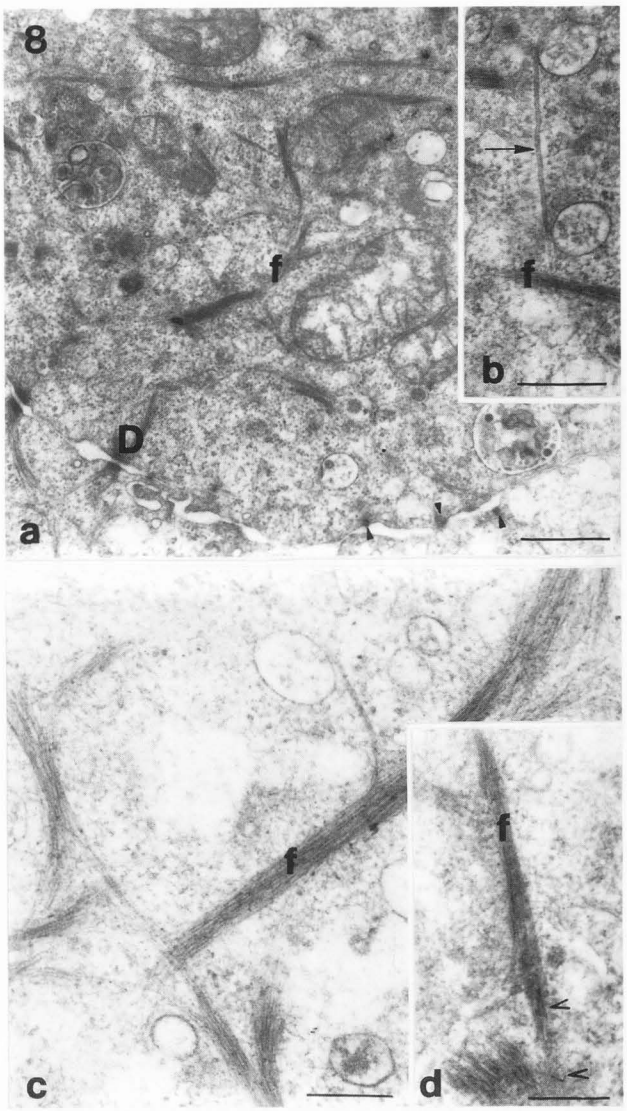

Figure 7. Electron micrograph of control HT29 cells. N: nucleus; f: filament bundles: →: nuclear pore: D: desmosome, <: microtubule. Bars = 1 μm (in a); 0.6 μm (in b and d) and 0.25 μm (in c).

Figure 8. Electron micrographs of cells X-irradiated with 1 Gy, 1 hour (**a** and **b**), and with 1 Gy, 24 hours (**c** and **d**). f: filament bundles; D: intact desmosome; ▸ = morphologically damaged desmosomes; →: microtubule. The filament bundle transformed into amorphous material (<). Bars = 0.8 μm (in a), 0.5 μm (in b), 0.3 μm (in c), and 0.25 μm (in d).

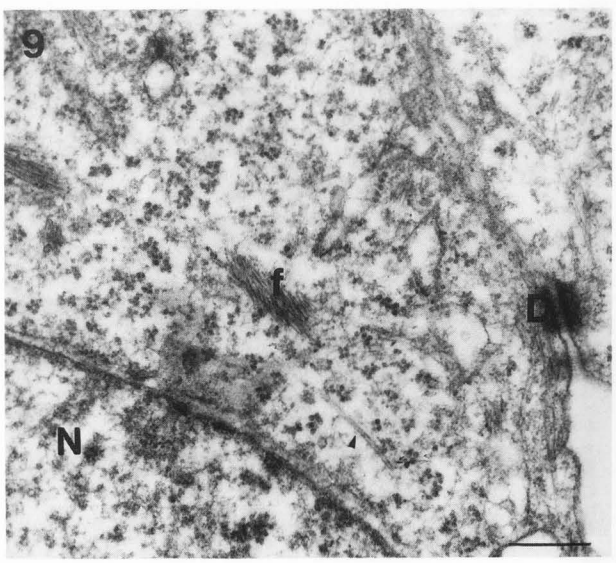

Figure 9. Electron micrograph of X-irradiated (1 Gy, 24 hours) HT29 cells. N: nucleus; f: filaments; d: desmosome; ▸: microtubule. Bar = 0.6 μm.

second antibody developed in goat (Sigma), diluted at 1:100, and applied on the sections for 60 minutes at room temperature. On some coverslips, the nuclei were counter-stained with 0.001% of ethydium bromide. The preparations were covered in PBS buffered glycerol (containing a few crystals of para-phenylene-diamine) and investigated under an Axioscope (Carl Zeiss, Jena, Germany). The photographs were taken on Kodakcolor negative roll films. It is especially difficult to produce high quality microphotographs in case of HT29 cells. They have columnar shape, even if they are cultured on glass surface; therefore, although the preparations were well focused, the fluorescence of the cytoskeletal network from the upper- and under-laying planes appears on negatives, too. The second difficulty is that the cytoskeleton is built by up much more thinner, delicate elements than, for example, in fibroblasts or other flat cell types. All photographs were taken at 1,000x.

Electron microscopy

The cells were fixed for 1 hour in 0.1 M phosphate buffered (2.5% glutaraldehyde, pH 7.3, 4°C), postfixed in 1% OsO_4 (pH 7.3 at 4°C for 1 hour), dehydrated with ethanol, and embedded in Durcupan (Fluka, Bucks, Switzerland). The samples were cut with diamond knives on an LKB Ultratome Nova (LKB, Uppsala, Sweden) and the sections were stained with lead citrate and uranyl acetate and examined in a JEOL (JEOL, Tokyo, Japan) 100CX transmission electron microscope.

Results

The microtubular system shows denser staining at the plasma membrane and some punctate staining in the cytoplasm (Fig. 1a). The filaments do not form cable-like rough bundles as they do in L929 fibroblasts (results not shown), but form very fine, thin, cotton-like network. Irradiation with 0.5 Gy and 1 Gy doses did not cause any changes of distribution of microtubules after 1 hour (Figs. 1b and 1c). One day after 1 Gy irradiation, the cells rounded up and we found more intensive tubulin staining in the cytoplasm between the nuclei and plasma membranes (Fig. 1d). Transmission electron microscopy did not show any radiation induced structural disorganization of microtubules, which were observable both in control and irradiated samples (shown later in Figs. 7c, 8b and 9).

Anti-vimentin gives filamental network in the cytoplasm of the untreated HT29 cells. Irradiation with 0.5 Gy for 1 hour did not exert any changes in its localization (Fig. 2b). However, 1 Gy after 1 and 24 hours, and 0.5 Gy after 24 hours caused the collapse of vimentin network (Figs. 2c and 2d). In these cases, vimentin could be detected as small spot-like aggregates (Figs. 2c and 2d).

Cytokeratin is assembled into a thin, delicate network of filaments in control cells, which surrounds the nucleus and extends towards the periphery of the cell (Fig. 3a). Soon after irradiation with either 0.5 or 1.0 Gy, this network begins to disrupt and clumps of cytokeratin accumulate in the cells. The punctuate pattern of cytokeratin distribution became very prominent 24 hours following irradiating with 1.0 Gy (Figs. 3b, 3c and 3d).

In control cultures, application of anti-actin resulted in a diffuse intracellular staining (probably due to the presence of short actin filaments). In addition, cable-like structures in the peripheral part of the cytoplasm (probably cortical actin filaments), and a few small actin aggregates were observed (Fig. 4a). Transformation of filamentous structures into spot-like aggregates was observed in cells 24 hours after irradiation (Figs. 4b and 4c).

In HT-29 cells, the distribution of vinculin does not show the well known punctuate pattern observed in fibroblasts, in which vinculin is associated with the focal contacts between the basal cell surface and the substratum. According to our observations, in control HT29 cells, the anti-vinculin antibody stains large bright areas relatively homogeneously (Fig. 5a). Irradiation caused a redistribution of vinculin to spoke-like structures, with large translucent patches between them (Figs. 5b, 5c and 5d).

Untreated HT29 cells display a punctuate pattern of staining when labeled with anti-desmosomal antibody. The dots of stain are localized at the periphery of the cells (Fig. 6a). Shortly after irradiation, the number of desmosome-like structures decreased. Twenty-four hours following irradiation, most of the cells rounded up and only a weak desmosomal protein staining could be observed on the surface of the separated cells (Figs. 6b and 6c).

Electron microscopy reveals intact desmosomes in control cells (Fig. 7d). Tonofilaments surround desmosomal plaques and extend between neighboring desmosomes. Long intermediate filament bundles were often found in association with desmosomes. A few normal and some of abnormal desmosomes were observed in the irradiated samples, they were characterized by the absence of tonofilaments and connecting intermediate filaments (Figs. 8a and 9). The perinuclear filament network (Figs. 7a and 7b) and cortical filaments (Fig. 7c) were easily detected by electron microscopy. Under the effect of irradiation, the perinuclear filaments and cortical filamental network almost disappeared from the cells and at the same time small bundles of filaments were formed irregularly in the cytoplasm (Figs. 8a, 8c and 9) associated with amorphous materials (Figs. 8a and 8d). These structural alterations persisted throughout the 24 hours observation period.

Discussion

The results reported here demonstrate that both the actin and IF based cytoskeletal systems of HT29 cells are sensitive targets of X-irradiation and respond to the injurious action of X-ray by transformation of filaments into clumps of dense aggregates of actin and/or IF proteins. The X-ray induced reorganization of the actin network into patches was observed earlier in cultured endothelial cells (Friedman *et al.*, 1986; Lin *et al.*, 1992; Kantak *et al.*, 1993) and in fibroblasts, too (Yanase *et al.*, 1993). However, signs of alteration of the actin filaments appeared within hours after irradiation, i.e., more rapidly than in the HT29 cells investigated in this study. These data suggest that the sensitivity of the actin-based cytoskeleton to X-ray in various cell types is different.

The exposure of cells to ionizing radiation modulate the expression of the actin gene (Woloschack and Chang-Liu, 1991; Lin *et al.*, 1992), and of microfilament protein expression in lung (Kasper *et al.*, 1993 a,b), squamous cell carcinomas (Fischer *et al.*, 1989), inner ear (Anniko *et al.*, 1989), and rat brain (Nedzvetskii *et al.*, 1990).

It is well known that some drugs and agents, including cytochalasins (Brett and Godman, 1986; Pasdar *et al.*, 1992; Stevenson and Begg, 1994), microtubular poisons (Pasdar *et al.*, 1992; Trevor *et al.*, 1995), tumor promoting factors (Shiba *et al.*, 1987; Grant and Aunis, 1990; Rahilly and Fleming, 1992), bacterial toxins (Malorni *et al.*, 1990), tumor necrosis factor (Lin *et al.*, 1992), interleukin (Bodo *et al.*, 1992), acrylamide (Gall *et al.*, 1992), etc., can induce reorganization and alteration of IFs in a variety of cells. Here we showed that X-irradiation is also a potent inducer of redistribution of IF proteins (cytokeratin, vimentin) and the actin associated protein, vinculin.

The integrity and assembly/disassembly of the cytoskeletal network is regulated by the cytosolic Ca^{++} level (Green *et al.*, 1987; Stevenson and Begg, 1994) and is modulated by phosphorylation/dephosphorylation of its proteins (Lamb *et al.*, 1989; Mays *et al.*, 1994). It is well known the ionizing radiation disturbs the Ca^{++} homeostasis of the cells (Somosy *et al.*, 1993, 1994; Zhivotovsky *et al.*, 1993) and induces changes in the intracellular level of cyclic nucleotides and influences the activity of protein kinase C (Somosy *et al.*, 1988; Hallahan *et al.*, 1991; Kandasamy and Harris, 1992). It seems plausible to propose that all of these regulatory mechanisms influencing the phosphorylation of proteins are related to the observed disruption of the cytoskeleton upon irradiation, although the exact mechanism of this phenomenon needs further elucidation.

One of the most conspicuous events found in this study was the loosening of cell contacts and the concomitant reduction in the number of desmosomes in irradiated cells. Since the IFs are obligate components of desmosomes, the disruption of the cytoskeletal network may destabilize the desmosomal contacts. There are reports in the literature showing that experimental disorganization of cytoskeletal filaments leads to labilization of desmosomes and loss of their function (Pasdar *et al.*, 1992).

Unlike actin and cytokeratin filaments, the microtubules have been shown to be insensitive to irradiation in HT29 cells. The relative radioresistance of the microtubules was also observed in endothelial cells and lymphocytes (Rubin *et al.*, 1988; Raicu *et al.*, 1993). Further studies are needed to elucidate the mechanism causing divergent sensitivity of the three kinds of cytoskeletal filaments to X-irradiation.

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References

Amos LA (1995) The microtubule lattice- 20 years on. Trends Cell Biol. **5**, 48-51.

Anniko M, Thornell LE, Hultcrantz M, Virtanen I, Ramaekers FC, Stigbrand T (1989) Prenatal low-dose gamma irradiation of the inner ear induces changes in the expression of intermediate filaments. Acta Otolaryngol. (Stockh). **108**, 206-216.

Barrit GJ (1992) Communication Within Animal Cells. Oxford University Press, New York. pp. 28-32.

Bodo M, Baroni T, Calvitti M, Carinci F, Arena N, Venti G (1992) Modulation of phenotype and cytoskeleton architecture by interleukin-1 alpha in human osteoblast-like cells *in vitro*. Acta Anat. **145**, 156-161.

Brett JG, Godman GC (1986) Cytoskeletal organization affects cellular responses to cytochalasins: Comparison of a normal line and its transformant. Tissue Cell **18**, 175-199.

Cleveland DW (1995) NuMa: A protein involved in nuclear structure, spindle assembly, and nuclear reformation. Trends Cell Biol. **5**, 60-64.

Cornforth MN, Goodwin EH (1991) Transmission of radiation-induced acentric chromosomal fragments to micronuclei in normal human fibroblasts. Radiat. Res. **126**, 210-217.

Coulombe PA (1993) The cellular and molecular biology of keratins: Beginning a new area. Curr. Opin. Cell Biol. **5**, 17-29.

Cramer LP, Mitchison TJ, Theriot JA (1994) Actin-dependent motile forces and cell motility. Curr. Opin. Cell Biol. **6**, 82-86.

Evans ML, Graham MM, Mahler PA, Rasey JS (1986) Changes in vascular permeability following thorax irradiation in the rat. Radiat. Res. **107**, 262-271.

Fischer HP, Wallner F, Maier H, Weber K, Osborn M, Altmannsberger M (1989) Coexpression of intermediate filaments in squamous cell carcinomas of upper aerodigestive tract before and after radiation and chemotherapy. Lab. Invest. **61**, 433-439.

Fogh J, Trempe S (1975) New human tumor cell lines. In: Human Tumor Cells *In Vitro*. Fogh J (ed.). Plenum Publishing Corp., New York. pp. 115-141.

Friedman M, Ryan US, Davenport WC, Chaney EL, Strickland DL, Kwock L (1986) Reversible alterations in cultured pulmonary arterial endothelial cell monolayer morphology and albumin permeability induced by ionizing radiation. J. Cell Physiol. **129**, 237-249.

Gall L, DeSmedt V, Ruffrini S (1992) Co-expression of cytokeratins and vimentin in sheep cumulus-oocyte complexes. Alteration of intermediate filament distribution by acrylamide. Develop. Growth Differ. **34**, 579-587.

Garrod DR (1993) Desmosomes and hemidesmosomes. Curr. Opin. Cell Biol. **5**, 30-40.

Grant NJ, Aunis D (1990) Effects of phorbol ester on cytoskeletal proteins in cultured bovine chromaffin cells: Induction of neurofilament phosphorylation and reorganization of actin. Eur. J. Cell Biol. **52**, 36-46.

Green KJ, Geiger B, Jones JCR, Talian JC, Goldman RD (1987) The relationship between intermediate filaments and microfilaments before and during the formation of desmosomes and adherents-type junctions in mouse epidermal keratinocytes. J. Cell Biol. **104**, 1389-1402.

Hallahan DE, Virudachalam S, Sherman ML, Huberman E, Kufe DW, Weichselbaum RR (1991) Tumor necrosis factor gene expression is mediated by protein kinase C following activation by ionizing radiation. Cancer Res. **51**: 4565-4569.

Heins S, Aebi U (1994) Making heads and tails of intermediate filament assembly, dynamics and networks. Curr. Opin. Cell Biol. **6**, 25-33.

Hirokava N (1994) Microtubule organization and dynamics dependent on microtubule-associated proteins. Curr. Opin. Cell Biol. **6**, 74-81.

Hitt AL, Luna EJ (1994) Membrane interactions with the actin cytoskeleton. Curr. Opin. Cell Biol. **6**, 120-130.

Kandasamy SB, Harris AH (1992) Effect of prostaglandins, inositol 1,4,5-triphosphate, and phorbol esters on radiation induced decreases in calcium influx in rat brain synaptosomes. Radiat. Res. **131**, 43-46.

Kantak SS, Diglio CA, Onada JM (1993) Low dose radiation-induced endothelial cell retraction. Int. J. Radiat. Biol. 64, 319-328.

Kasper M, Rudolf T, Haase M, Schuh D, Müller M (1993a) Changes in cytokeratin, vimentin and desmoplakin distribution during the repair of irradiation-induced lung injury in adult rats. Virchows Arch. B. Cell Pathol. **64**, 271-279.

Kasper M, Rudolf T, Hahn R, Peterson I, Müller M (1993b) Immune- and lectin histochemistry of epithelial subtypes and their changes in a radiation-induced lung fibrosis model of the mini pig. Histochemistry **100**, 367-377.

Lamb NJC, Fernandez A, Feramisco, Welch WJ (1989) Modulation of vimentin containing intermediate filament distribution and phosphorylation in living fibroblasts by the cAMP-dependent protein kinase. J. Cell Biol. **108**, 2409-2422.

Law MP, Ahier RG (1989) Vascular and epithelial damage in the lung of mouse after X-rays and neutrons. Radiat. Res. **117**, 128-144.

Lawrence TS, Davis MA, Maybaum J (1994) Dependence of 5-fluorouracil-mediated radiosensitization on DNA-directed effects. Int. J. Radiat. Oncol. Biol. Phys. **29**; 519-523.

Lin PS, Ho KC, Sung SJ, Gladding J (1992) Effect of tumor necrosis factor, heat, and radiation on the viability and microfilament organization in cultured endothelial cells. Int. J. Hyperthermia **8**, 667-677.

Malorni W, Fiorentini C, Paradisi S, Giuliano M, Mastrantonio P, Donelli G (1990) Surface blebbing and cytoskeletal changes induced *in vitro* by toxin B from Clostridium difficile: An immunochemical and ultrastructural study. Exp. Molec. Pathol. **52**, 340-356.

Mays RW, Beck KA, Nelson JW (1994) Organization and function of the cytoskeleton in polarized epithelial cells: A component of the protein sorting machinery. Curr. Opin. Cell Biol. **6**, 16-24.

Nedzvetskii VS, Busygina SG, Berezin VA, Dvoterskii AL (1990) The CNS syndrome. The characteristics of the intermediate filaments of the rat brain. Radiobiologiia **30**, 243-246.

Pasdar M, Li Z, Kreminski KA (1992) Desmosome assembly in MDCK epithelial cells does not require the presence of functional microtubules. Cell Motil. **23**, 201-212.

Rahilly MA, Fleming S (1992) A tumor promoter induces alteration in vinculin and actin distribution in human renal epithelium. J. Pathol. **166**, 283-288.

Raicu M, Vral A, Thierens H, De Ridder L (1993) Radiation damage to endothelial cells *in vitro*, as judged by the micronucleus assay. Mutagenesis **8**, 335-339.

Reisler E (1993) Actin molecular structure and function. Curr. Opin. Cell Biol. **5**, 41-47.

Rubin DB, Drab EA, Waed WF, Bauer KD (1988) Cell cycle progression in irradiated endothelial cells cultured from bovine aorta. Radiat. Res. **116**, 364-371.

Shiba Y, Sastrodihardjo S, Sasaki Y, Kanno Y (1987) Intercellular contacts and the organization of actin filaments in cultured epithelial FL cells are altered by growth on type I collagen and treatment with 12-O-tetradecanoylphorbol-13-acetate through the modulation of interactions between cells and the substratum. Cell Struct. Funct. **12**, 549-558.

Small JV (1995) Getting the actin filaments straight: Nucleation-release or tread-milling. Trends Cell Biol. **5**, 52-55.

Somosy Z, Kubasova T, Köteles GJ (1987) The effects of low doses of ionizing radiation upon the micromorphology and functional state of cell surface. Scanning Microsc. **1**, 1267-1278.

Somosy Z, Takáts A, Kubasova T, Sudár F, Köteles GJ (1988) Effects of X-irradiation on adenylate cyclase activity and cyclic AMP content of primary human fibroblasts. Scanning Microsc. **2**, 2165-2172.

Somosy Z, Kovács J, Siklós L, Köteles GJ (1993) Morphological and histochemical changes in intercellular junctional complexes in epithelial cells of mouse small intestine upon X-irradiation: Changes of ruthenium red permeability and calcium content. Scanning Microsc. **7**, 961-971.

Somosy Z, Thuróczy G, Köteles GJ, Kovács J (1994) Effects of modulated microwave and X-ray irradiation on the activity and distribution of Ca^{2+}-ATPase in small intestine epithelial cells. Scanning Microsc. **8**. 613-620.

Stevenson BR, Begg DA (1994) Concentration-dependent effects of cytochalasin D on tight junctions and actin filaments in MDCK epithelial cells. J. Cell Sci. **107**, 367-375.

Stewart M (1993) Intermediate filament structure and assembly. Curr. Opin. Cell Biol. **5**, 3-11.

Trevor KT, McGuire JG, Leonove EV (1995) Association of vimentin intermediate filaments with the centrosome. J. Cell Sci. **108**, 343-356.

Ungar F, Geiger B, Ben-Ze'ev A (1986) Cell contact- and shape-dependent regulation of vinculin synthesis in cultured fibroblasts. Nature **319**, 787-791.

Wallee RB, Okamoto PM (1995) The regulation of endocytosis: Identifying dynamin's binding partners. Trends Cell Biol. **5**, 43-47.

Woloschack GE, Chang-Liu CM (1991) Expression of cytoskeletal elements in proliferating cells following radiation exposure. Int. J. Radiat. Biol. **59**, 1173-1183.

Woloschack GE, Shearin-Jones P, Chang-Liu CM (1990) Effects of ionizing radiation on expression of genes encoding cytoskeletal elements: Kinetics and dose effects. Mol. Carcinog. **3**, 374-378.

Yanase A, Ueda M, Kaneda T, Torii S, Matsuyama M (1993) Irradiation effects on wound contraction using a connective tissue model. Ann. Plast. Surg. **30**, 435-440.

Yau TM (1981) Alterations in the structure and function of the mammalian cell membrane after exposure to ionizing radiation. Scanning Electron Microsc. **1981**; IV: 47-54.

Zhivotovsky B, Nicotera P, Bellomo G, Hanson K, Orrenius S (1993) Ca^{2+} and endonuclease activation in radiation induced lymphoid cell death. Exp. Cell Res. **207**, 163-170.

Discussion with Reviewers

M. Kasper: There is some evidence that irradiation induces synthesis of inflammatory gene products such as certain transcription factors, for example, c-fos and c-jun. Target genes for these factors are also the genes encoding keratins. What about the distribution of c-fos and c-jun in cells after disassembly of filaments?

Authors: We did not investigate the subcellular distribution of these factors upon irradiation and we do not have any data about it.

P.B. Bell: Considering previous report that osmium disrupts actin filaments, why did you use osmium postfixation and what precaution did you take to prevent damage?

Authors: According to our observations, the osmium postfixation does not disturb essentially the conventional ultrastructure of cortical actin filaments, because they remained intact in control HT29 cells (Fig. 7c), but this structure was disorganized on the irradiated preparations.

P.B. Bell: What is the explanation of the diffuse vinculin pattern in control cells and its change to a spot-like arrangement after irradiation?

Authors: We think the vimentin filaments depolarized upon irradiation and formed clumps. Similar, depolymerization-induced clump formation are known in case of other kinds of cytoplasmic filaments as we briefly reviewed in the text.

P.B. Bell: What evidence is there that irradiation exerts it effects on the cytoskeleton indirectly through its effects on calcium, rather than through other direct or indirect mechanisms?

Authors: The integrity of cytoskeletal elements is dependent on phosphorylation of their subunits by certain kinases, and in many cases, phosphorylation facilitates the disassembly of cytoskeletal filaments as reviewed by Eriksson *et al.* (1992). The low doses of irradiation can

activate the protein kinase C enzyme probably via elevated cytoplasmic calcium content and/or modify cAMP content (which can activated other kinases) (Somosy *et al.*, 1988; Hallahan *et al.*, 1991). It is known that the microtubules are very radioresistant in cell free system, i.e., as reported by Coss *et al* (1981), inhibitory effect on microtubule assembly were found only upon 5,000 to 30,000 rad X-radiation doses. Considering these data, it is obvious to suppose the indirect effect(s) of irradiation to this system.

L.S. Yasui: What is the biological significance of your results?
Authors: Our data give new direct evidences on the role of cytoskeletal system in some irradiation induced morphological alterations, i.e., changes of cell shape, cell to cell contacts, distribution of cell organelles, as well as radiation induced redistribution of different cytoskeletal elements.

L.S. Yasui: Why were confluent cultures of adenocarcinoma cells used for this experiment? Has the radiation response of the cytoskeleton not been documented in many cell lines and how do these other results correlate with your findings?
Authors: We used confluent cultures of HT29 cells; in our experiment only this cell type was used. However, there are direct or indirect data, as we listed in the text, on similar radiation induced cytoskeletal changes in other cell types.

M. Malecki: Have you noticed any differences in cell morphology, or cell attachments, or cell contact between cells grown on glass as compared to those on Thermanox?
Authors: We did not find any differences in cell morphology, cell attachments and cell contacts between cells grown on glass and Thermanox.

M. Malecki: What was percentage of HT29 cells suffering from the symptoms you describe?
Authors: The radiation-induced and described distribution changes of investigated cytoskeletal elements were detected in approximately 90% of cells.

M. Malecki: Are the results of clonogenic assays or synchronized cultures available?
Authors: No, we used only non-synchronized cultures of HT29 cells.

M. Malecki: Can you explain striking differences between previously published patterns of vinculin or cytokeratin in normal or transformed cultured cells (Lehto *et al.*, 1982; David-Pfeity T and Singer SJ, 1980; Tarone *et al.*, 1985; Franke *et al.*, 1978; Moll *et al.*; etc.) and patterns of these proteins obtained your work?
Authors: As reported in the literature, the vinculin is usually localized at the adhesion plaques of fibroblasts and other cell types. However, diffuse vinculin distribution in certain tumor cells were also found (Sadano *et al.*, 1992). We observed dotted distribution of vinculin in L929 fibroblast similar to literature data. The reported vinculin pattern in HT29 cells may be explained at least by two items: (1) this cell line is of carcinoma origin, and (2) we investigated this cell type in undifferentiated form.

The observed cytokeratin pattern is in agreement with other data which is known about HT29 cells (Plateroti *et al.*, 1993).

M. Malecki: Could you comment on the effects of X-irradiation on the proliferation rate-doubling time? Did the affected cells recover after or did they die as the result of this X-irradiation? What was the survival rate? What were the viability tests?
Authors: We did not investigated the effects of X-irradiation on the proliferation rate-doubling time and recovery of radiation-induced effects of cytoskeletal elements. However, the survival rate of HT29 cells were known from literature (Lawrence *et al.*, 1994). These data suggest that the irradiation doses used (0.5 and 1 Gy) do not cause cell death during the studied period (24 hour), this was confirmed with trypan blue test.

Additional References

Coss RA, Bamburg JR, Dewey WC (1981) The effects of X-irradiation on microtubule assembly *in vitro*. Radiat. Res. **85**, 99-115.

David-Pfeity T, Singer SJ (1980) Altered distributions of the cytoskeletal proteins vinculin and α-actinin in cultured fibroblasts transformed by Rous sarcoma virus. Proc. Natn. Acad. Sci. U.S.A. **77**, 6687-6691.

Eriksson JE, Puneet O, Goldman RD (1992) Intermediate filament dynamics. Curr. Opin. Cell Biol. **4**, 94-98.

Franke W, Schmidt E, Osborn M, Weber K (1978) Different intermediate-sized filaments distinguished by immunofluorescence microscopy. Proc. Natn. Acad. Sci. U.S.A. **75**, 5034-5038.

Lehto VR, Hovi T, Vartio T, Badley RA, Virtanen NI (1982) Reorganization of cytoskeletal and contractile elements during transition of human monocytes into adherent macrophages. Lab. Invest, **47**, 391-399.

Moll R, Franke W, Schiller P, Geiger M, Krepler R (1982) The catalog of human cytokeratins: Patterns of expression in normal epithelia, tumors and cultured cells. Cell **31**, 11-24.

Plateroti M, Sambuy Y, Nobili F, Bises G, Perozzi G (1993) Expression of epithelial markers and retionid-binding proteins in retinol- or retinoic acid-treated intestinal cells *in vitro*. Exp. Cell Res. **208**, 137-147.

Sadano H, Inoue M, Taniguchi S (1992) Differential expression of vinculin between weakly and highly metastatic B16-melanoma cell lines. Jpn. J. Cancer Res. **83**, 625-630.

Tarone G, Cirillio D, Graneotti FG, Comolio PM, Marchisio PC (1985) Roues sarcoma virus-transformed fibroblasts adhere primarily at discrete protrusions of the ventral membrane called podosomes. Exp. Cell Res. **159**, 141-157.