Delayed Growth Suppression and Radioresistance Induced by Long-Term Continuous Gamma Irradiation

Hiroo Nakajima, Chiharu Furukawa, Young-Chae Chang, Hiromitsu Ogata, and Junji Magae

Department of Breast Surgery, Misugi-kai Sato Hospital, 65-1 Yabuhigashi-machi, Maikata-shi, Osaka 573-1124, Japan; Department of Biotechnology, Institute of Research and Innovation, 1201 Takada, Kashiwa 277-0861, Japan; Department of Cell Biology, Catholic University of Daegu, School of Medicine, 3056-6 Daemyung-4-Dong, Nam-gu, Daegu 705-718, Republic of Korea; Center for Information Research and Library, National Institute of Public Health, 2-3-6 Minami, Wako, Saitama 351-0197, Japan; Magae Bioscience Institute, 49-4 Fujimidai, Tsukuba 300-1263, Japan; and Radiation Safety Research Center, Nuclear Technology Research Laboratory, Central Research Institute of Electric Power Industry, 2-11-1 Iwado Kita, Komae, Tokyo 201-8511, Japan

INTRODUCTION

The biological effects of radiation depend not only on radiation type and total dose, but also on radiation dose rate and duration, and many investigators have reported differences between the effects of acute and chronic irradiation. A direct dose-rate effect, or dose-rate sparing effect, has been reported for various endpoints including growth arrest, oncogenesis, mutation and chromosome instability (1-10). It is believed that the dose-rate effect occurs because living organisms have developed mechanisms for attenuating the toxic effects of radiation. For instance, reactive oxygen species generated by radiation are detoxified by antioxidant enzyme systems (11), and DNA lesions are repaired by specific protein complexes (12). Stress-related proteins are induced or activated in irradiated cells, controlling cellular damage (13, 14). Organisms can protect themselves from radiation-induced toxicity if they have sufficient time to counteract radiation-induced intracellular reactions.

On the other hand, some investigators have presented evidence that radiation causes an inverse dose-rate effect in which low-dose-rate radiation induces a more significant biological impact than higher dose rates on survival (15), mutation (16), micronucleus formation (17) and transformation (18, 19) by fission neutrons and ionizing radiation. Such hypersensitivity to lower dose rates is in part explained by differences in radiosensitivity among cell cycle phases (15). Low-dose-rate irradiation over a longer time period increases the proportion of cells exposed to radiation in G2 phase, the most radiosensitive phase in the cell cycle. Recently it has been suggested that there is a threshold dose for induction of the repair system for DNA double-strand breaks (DSBs) (20). That is, DSBs accumulate in cells without repair of the lesion until the number of intracellular DSBs reaches a threshold level. Cells with accumulated DSBs are eliminated by apoptosis if they pass through mitosis. This hypothesis predicts that radiation-induced damage accumulates at a dose rate below the threshold level that can induce the DSB repair system. Further, it has been reported that DNA damage sensor (ataxia telangiectasia mutated; ATM) activation is attenuated in cells exposed to low-dose-rate gamma (γ) rays, and...
survival rate is inversely related to dose rate in human fibroblasts (21).

Biological responses to low-dose/low-dose-rate radiation occur through relatively subtle effects, often mediated by molecular signaling, that result in a modification of an organism’s subsequent biochemical response to radiation. The effects of low-dose/low-dose-rate ionizing radiation, which can positively or negatively modulate the toxicity of radiation, include adaptive response, bystander effect, radiation hormesis and low-dose hypersensitivity (22). Radiation-induced adaptive responses are the attenuation of radiation toxicity through pretreatment of cells or animals with low-dose/low-dose-rate radiation (22, 23). Adaptive responses, usually observed at doses above 5 mGy and below 200 mGy, can reduce radiation-induced DNA damage, mutagenesis, chromosomal aberrations, micronuclei and cell transformation. The bystander effect occurs in cells not directly exposed to radiation by signaling between irradiated and nearby nonirradiated cells through gap junctions, cognate interaction of cell surface molecules or soluble factors secreted by the irradiated cells (22, 23). The phenomenon of low-dose radiation hypersensitivity, the exaggerated sensitivity of cells to very low doses of ionizing radiation observed at a few hundred mGy per dose increment, is a response specific to G2-phase cells, presumably due to ineffective cell cycle arrest and DNA repair in G2-phase cells (22, 24). These low-dose/low-dose-rate responses are clearly nonlinear radiation responses that depend on the nature of radiation, cell and tissue type, the genetic and physiological status of the cells and the microenvironment of tissue.

Radiation therapy is a powerful strategy for cancer treatment, however, as in chemotherapy, recurrent tumor cells that survive initial therapy are resistant to secondary radiation therapy as well as to conventional chemotherapy (25). This resistance is often observed in fractionated irradiation regimens, designed to prevent damage to normal tissues surrounding the tumor, in which all tumor cells are not eliminated by any single irradiation. Activation of Src and downstream p38 MAPK and protein kinase B by irradiation promotes epithelial-mesenchymal cell transition and a drug-resistant breast cancer cell phenotype (26). Loss of the ATM/Chk2/p53 pathway that is activated in response to DNA damage and arrests cells in G2/M phase in normal tissues accelerates tumor development and contributes to radioresistance in gliomas (27).

Alternatively, sublethal-dose or chronic irradiation has ameliorative effects in certain contexts (28, 29). We observed that chronic γ-ray irradiation improves glucose clearance in db/db mice, an experimental model for type II diabetes, by maintaining insulin secretion, which gradually decreases during the progression of diabetes due to degeneration of pancreatic islets (30). Low-dose-rate γ-ray irradiation from ten weeks of age throughout the lives of these mice ameliorates diabetic nephropathy, moderates kidney complications and prolongs lifespan (31). Continuous low-dose-rate γ-ray irradiation also prolongs lifespan in a mouse model for accelerated aging due to a mutation in Klotho (32). Protection from oxidative damage evoked by radiation-activated stress response has been implicated in these ameliorative effects. These observations suggest that the irradiation schedule is critical to obtaining the desirable effects of radiation in protection and therapeutic use.

We previously proposed the modified exponential (MOE) model for evaluating dose-rate effects, constructed through the statistical analysis of [3H]thymidine uptake and micronucleus formation in human osteosarcoma cells exposed to an array of doses, dose rates and irradiation times (33, 34). The MOE model describes the relationship between the logarithm of the median effective dose (MED) and dose rate, allowing evaluation of efficacy over a wide range of doses and dose rates. The MOE model demonstrates that, at low dose rates, a direct dose-rate effect increases exponentially as dose rate decreases or exposure time increases, while no dose-rate effect appears and radiation risk is solely determined by dose at relatively higher dose rates of more than 1 Gy/h. Further study has demonstrated that the MOE model accurately describes the dose-rate effect observed in cells with an intact DSB repair system during the early phase of continuous γ-ray exposure. There also exists a distinct dose-rate effect mechanism that is clearly observed in longer time exposures of more than 1,000 h, determined solely by dose rate, but not by total dose, and independent of the DSB repair system involving DNA-dependent protein kinase or ATM (35).

c-Jun is one of the proteins that make up the transcription factor activator protein-1 (AP-1). AP-1 has been implicated in the transcriptional regulation of a wide range of genes participating in cell survival, proliferation, oncogenesis and apoptosis (36, 37). AP-1 was identified as a transcription factor that contributes both to basal gene expression and tetradecanoyl-phorbol-acetate (TPA)-inducible gene expression. AP-1 is activated by various stimuli such as growth factors, pro-inflammatory cytokines and genotoxins, including radiation. In this study, we further examined the nature of growth inhibitory effects caused by continuous irradiation and found that human cell lines exposed to continuous low-dose-rate γ rays have properties distinct from those exposed to acute high-dose rates. These cells acquire a resistance to apoptosis that correlates to expression of c-Jun. These results suggest that dose-rate efficacy should be reevaluated to prevent radiation-induced malignancy in clinical therapy and to obtain optimal ameliorative effects in the biological use of radiation.

MATERIALS AND METHODS

Cell Cultures

A human osteosarcoma, U2OS, was kindly provided by N. H. Heintz (University of Vermont, Burlington, VT). A human glioma cell line, M059K, was purchased from ATCC® (Manassas, VA). These cells were cultured at 37°C in a 10% CO2 atmosphere in D-MEM.
supplemented with 5% fetal bovine serum, 50 μg/ml kanamycin and 8 μg/ml tylosin.

Irradiation Procedures

For continuous irradiation over several months, cells in a 25-cm² culture flask containing 5 ml media were cultured at 37°C in 10% CO₂ atmosphere in a CO₂ incubator placed in the irradiation room with a 1.85 PBq cobalt-60 (⁶⁰Co) source (33, 35). The cells were maintained in exponential growth conditions at a cell number between 5 × 10⁵ and 5 × 10⁶ cells/bottle by transferring cultures into fresh media twice per week. In each transfer, cells were washed with 0.25% trypsin (Sigma-Aldrich®, LLC, St. Louis, MO) in PBS, briefly incubated at 37°C and suspended in fresh media to suppress protease activity. Half-a-million cells were transferred to a 25-cm² culture flask containing 5 ml media and cultured for three or four days before the next transfer. For acute irradiation of several minutes, cells in a 25-cm² culture flask containing 5 ml media for cell cycle analysis, in a 2-cm diameter culture dish containing 2 ml media for [³H]thymidine incorporation assay and sample preparation for Western blotting, were cultured for 18 h to allow cells to settle down to the bottom, and exposed to γ rays in the irradiation room, or X rays operated at 150 kV, with a 5 mA tube current and a 1.0 mm aluminum plus 0.2 mm copper filter (MBR-1505R2; Hitachi Ltd., Tokyo, Japan), at room temperature for the indicated times immediately after removal from the CO₂ incubator. Dose rate was measured with a photoluminescent dosimeter (Asahi Technoglass Co., Funabashi, Japan) (38).

[³H]Thymidine Incorporation

Single cell suspension was achieved by trypsin treatment, and 5,000 cells were cultured for 48 h in a quadruplicate microplate well containing 0.2 ml culture media, followed by a 4-h pulse of 0.5 μCi/well [³H]thymidine before harvesting on a glass filter (33, 35). To harvest adherent cells, cells were washed with PBS and detached from the bottom of the well by trypsinization for 10 min. Incorporated radioactivity was measured by liquid scintillation counting.

Cell Cycle Analysis

One million cells were suspended in staining buffer consisting of 50 μg/ml propidium iodide, 0.2% Nonident™ P-40 and 4 mM sodium citrate (39). The fluorescence of stained nuclei was analyzed by flow cytometry (Coulter® Epics® XL™; Brea, CA).

Western Blot Analysis

Cell lysates were prepared by suspending cells in 2× SDS sample buffer [120 mM Tris, 4% SDS, 20% glycerol, 0.1 mg/ml bromophenol-blue and 100 mM dithiothreitol (pH 6.8)] at a density of 1 × 10⁶ cells/ml. After a brief sonication, the lysates were heated at 95°C for 5 min. The proteins were electrotransferred to Immobilon-P membranes (Millipore, Darmstadt, Germany). Western blotting of all samples was performed as described previously (39) with first antibodies and the corresponding second antibodies for whole immunoglobulins from mouse or rabbit (Amersham Biosciences, Buckinghamshire, UK). Specific protein detection was performed with an enhanced chemiluminescence Western blotting kit (Roche Diagnostics, Mannheim, Germany), following the manufacturer’s instructions. Specific antibodies for Tp53 (DO-1), p38 (A-12), and phospho-Tyr204-Erk-1 (E-4) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-β-actin (C4) antibody was acquired from Abcam Ltd. (Cambridgeshire, UK). Antibodies for phosphorylated forms of Ser73 (cat. no. 9261) and Ser63 (cat. no. 9164) in c-Jun and Thr180/Tyr182 in p38 (cat. no. 9211) were acquired from Cell Signaling Technology® Inc. (Danvers, MA) and those for c-Jun (clone 3) and Erk-1 (clone MK12) were acquired from BD Transduction Laboratories™ (Lexington, KY) and PARP (Ab-2) was acquired from Calbiochem (Darmstadt, Germany).

RESULTS

Previously, we observed dose-rate effects on micronucleus formation and growth inhibition of human cell lines in which the effects declined exponentially as the dose rate decreased, and proposed a new dose-rate effect model, the “MOE model”, through statistical analysis of that data (33, 34). In this work, we studied properties of human osteosarcoma (U2OS) and glioma (M059K) cell lines exposed to low-dose-rate radiation for more than five months. U2OS has wild-type p53 (40), while M059K has mutant p53 defective in DNA binding ability (41). Unlike normal cell lines that gradually alter growth rate and the other properties with the progression of senescence, tumor cell lines maintain growth properties consistently throughout the year-long experiment. Cells were exposed continuously to γ rays in an irradiation room containing a 1.85 PBq ⁶⁰Co source. Growth inhibition was equilibrated to depend solely on dose rate at the end of continuous irradiation over a period of several months (35). Continuous irradiation for 222 days at 53.6 mGy/h (total dose, 286 Gy) reduced [³H]thymidine uptake to 31.8% in M059K cells and to 9.5% in U2OS cells, and almost no inhibitory effect was observed at dose rates lower than 4 mGy/h. The MED increased as irradiation duration increased, while the dose rate that produces the MED was consistent at approximately 20 mGy/h when cells were irradiated for longer than 127 h. We were unable to maintain cells at dose rates greater than 100 mGy/h for exposures longer than one month. Acute irradiation for 2.4 min significantly inhibited cell growth at doses higher than 1 Gy and [³H]thymidine uptake was reduced to 24% in M059K cells and to 6% in U2OS cells after 12 Gy (310 Gy/h) irradiation (Fig. 1A). Cell cycle analysis showed that chronic irradiation at 53.6 mGy/h for 222 days (total dose, 286 Gy) did not produce the accumulation of G₂-phase cells, which was evident in both cell lines exposed to acute high-dose-rate X rays (22.9 Gy/h) at doses over 5 Gy (Fig. 1B). These results suggest that acute and chronic irradiation produce entirely different inhibitory effects on cell growth.

Growth suppression by chronic irradiation at 53.6 mGy/h for more than 150 days was cytostatic, and no population with sub-G₁ DNA content was detected in either cell line. This suggests that growth suppression caused by chronic irradiation is reversible. To test this inference, we further cultured cells after the 222-day chronic irradiation under a background dose-rate environment (0.055 μGy/h). Unexpectedly, both cell lines irradiated with chronic low-dose-rate γ rays maintained growth retardation for four weeks after irradiation was stopped. It took more than five weeks for U2OS cells to gradually recover their normal growth rate. M059K cells maintained growth suppression up to eight weeks after terminating exposure (Fig. 2).
Delayed growth suppression is one of the delayed effects of ionizing radiation that is assumed to be associated with genetic instability (42, 43), an accumulation of oncogenic mutations and malignant properties (22, 44, 45). It is known that the cytotoxic effect of radiation occurs mainly through reproductive cell death caused by mitotic failure of cells with incompletely repaired DNA strands. The cytostatic cells under chronic irradiation that accumulate in G1 phase would be relatively resistant to such reproductive death or apoptosis, which may contribute to the acquired malignancy of cancer cells after radiation treatment. This supposition prompted us to study the resistance of these cells to chemotherapeutic agents. Although we could not find any apoptosis in U2OS cells exposed to various apoptosis-inducing agents, M059K cells underwent apoptosis, as evidenced by poly(ADP ribose) polymerase (PARP) cleavage, a typical marker for activation of effector caspases associated with apoptosis (46), upon exposure to DNA damaging agents such as ultraviolet (UV) radiation, cisplatin, doxorubicin or etoposide, as well as staurosporine, a protein kinase inhibitor (Fig. 3). It should be noted that the apoptosis is p53-independent because M059K cells have a mutation in p53 (41). The apoptosis induced by these treatments was substantially attenuated in cells continuously exposed to radiation for 222 days at 53.6 mGy/h (286 Gy total dose). Cells were further cultured for 48 h under the background dose rate, and the cell cycle profile was analyzed by flow cytometry.
mGy/h. UV-radiation exposure caused evident apoptosis in controls at 8 h postirradiation, while cells continuously exposed to low-dose-rate γ rays underwent only slight apoptosis even 17 h after UV irradiation. Cisplatin and doxorubicin caused evident apoptosis in controls at 8 h postirradiation, while cells irradiated chronically underwent apoptosis at 17 h postirradiation. Etoposide induced apoptosis in controls as early as 4 h postirradiation, but chronically exposed cells underwent apoptosis to a similar extent as late as 17 h after etoposide treatment. Staurosporine induced complete apoptosis in controls at 8 h after treatment, but produced only partial apoptosis in chronically irradiated cells. These results suggest that chronic irradiation endows cells with resistance to apoptosis-inducing agents, and may contribute to radioresistance in tumor cells.

It is suggested that the delayed expression of cell death is an induced epigenetic alteration of gene expression that is related to radiation-induced stress responses, including

![Graph showing delayed growth suppression by continuous irradiation.](image)

**FIG. 2.** Delayed growth suppression by continuous irradiation. M059K (open circles) or U2OS cells (closed circles) were γ-ray irradiated for 222 days with 53.6 mGy/h. Cells were further cultured at a background dose rate for the time indicated (recovery time). At the end of the culture period, cells were trypsinized to detach them from the culture dish and further cultured in a micro-plate well for 48 h. [3H]thymidine uptake of four independent cultures was determined 4 h prior to harvesting. Average percentage of radioactivity compared to control cells cultured at the background dose rate is shown.

![Graph showing attenuation of apoptosis in M059K cells chronically exposed to γ rays.](image)

**FIG. 3.** Attenuation of apoptosis in M059K cells chronically exposed to γ rays. M059K cells were γ-ray irradiated for 222 days at 53.6 mGy/h. Cells were further cultured at the background dose rate for 30 days. After trypsin treatment to detach cells from the culture dish, one million of the cells (2 × 10⁵) in 2 ml media were cultured for 18 h, allowing them to settle, and further cultured for the times (in hours) indicated after the addition of a drug or UV exposure. For each time point indicated, cells were scraped from the dish, and PARP cleavage was visualized by Western blotting.
DNA-damage and oxidative stress responses (42, 43). The tumor suppressor p53, which induces downstream targets involved in radiation-induced apoptosis, is the transcription factor affected most profoundly by ionizing radiation, and the level of p53 expression has been found to change with time after irradiation (47). In our study, Western blotting showed a higher level of p53 expressed in M059K cells than in U2OS cells, and the level of p53 remained unchanged in M059K cells after chronic irradiation, even after culturing for 30 days at a background dose rate (Fig. 4). In contrast, p53 was induced in U2OS cells at a level comparable to that observed in M059K immediately after chronic irradiation, and that level decreased after culturing for 30 days at background dose-rate conditions. Similarly, high-level expression of c-Jun was sustained for 30 days after termination of exposure, in parallel with growth suppression. Phosphorylated forms of c-Jun were also elevated in chronically irradiated cells, as well as in cells cultured at a background dose rate for 30 days after termination of chronic irradiation. This activation was not observed in MAPK family proteins p38 and Erk, suggesting that the effect on c-Jun is specific and highly correlated to delayed growth suppression in both cell lines.

The phosphorylation of c-Jun at serine 15 in M059K cells was dramatically increased by acute 5 Gy X-ray irradiation and reversed to the control level after culturing in background dose-rate conditions for 30 days (Fig. 5A). Conversely, expression of c-Jun and its phosphorylation at serine 63 was only slightly increased by acute irradiation and reversed to the control level after 30-day culturing in a background dose-rate environment. Sensitivity to doxorubicin-induced apoptosis did not change in cells cultured for 30 days after acute irradiation (Fig. 5B). These results suggest that delayed effects such as activation of c-Jun and resistance to apoptosis are characteristics of cells that maintain suppressed proliferation throughout a prolonged culturing period in a low-dose-rate environment after a long period of acute irradiation.

**DISCUSSION**

In this study, we observed that growth inhibition in continuously irradiated cells was cytostatic and character-
induces premature senescence, the accumulation of G₁-radiation-induced continuous stress. Although radiation arrest, residual unrepaired DSBs remain (41). Thus, M059K lacks G₁-phase-checkpoint and p53 accumulation after ionizing irradiation (50).

Another property we observed in chronically irradiated cells is delayed phenotype. The growth retardation we observed in chronically irradiated cells persisted for more than one month after termination of exposure. Like genetic instability, delayed growth suppression is one of the nontargeted effects of radiation that is assumed to lead indirectly to carcinogenesis (42–45). Delayed expression of cell death or lethal mutation is conventionally detected by clonogenic survivors in a cell population by colony formation assay and occurs predominantly from apoptosis. We detected growth retardation by counting cells or by [³H]thymidine uptake of cells in bulk culture. In contrast to radiation-induced lethal mutational, flow cytometry analysis revealed no apoptotic cell population in the sub-G₁ region. Thus, delayed expression of cytostatic growth retardation might be a characteristic of long-term chronic irradiation. We found that cells with growth retardation induced by chronic irradiation were resistant to apoptosis induced by various apoptosis-inducing agents such as UV, genotoxins and staurosporine, suggesting that chronic irradiation endows cells with chemotherapy resistance. Thus, the radioreistance that can occur after repeated exposures allow tumor cells to survive after treatment diminishes the effectiveness of radiation (25). Repeated radiation exposures may induce malignant phenotypes through the cytostatic survival of irradiated cells.

To identify the biochemical events that might cause cytostatic growth retardation, we compared the expression of stress-related proteins induced by DNA damage or by oxidative stress. p53 was significantly activated in U2OS cells immediately after the termination of chronic irradiation, and it gradually decreased to the control level within one month after the termination of irradiation. Acute irradiation induced p53 phosphorylation in M059K with minimum activation of c-Jun. This p53 activation was diminished one month after the termination of irradiation. Thus, delayed expression of cytostatic survival of irradiated cells.

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FIG. 5. Delayed effects on M059K cells acutely exposed to X rays. M059K cells were X-ray irradiated with 5 Gy (13.1 min at 22.9 Gy/h). The cells were further cultured at the background dose rate for 30 days. The cells were harvested, and protein expression was analyzed directly by Western blotting (panel A), or treated with trypsin to detach cells from the culture dish. One million of these detached cells (2 × 10⁵) in 2 ml media were cultured for 18 h, allowing them to settle, and further cultured for the times (in hours) indicated after the addition of 3 μM doxorubicin. For each time point indicated, cells were scraped from the dish, and PARP cleavage was visualized by Western blotting (panel B).
responding factors such as Erk and p38 were hardly affected (Fig. 4). Similarly, it has been proposed that nontargeting effects of radiation, including genetic instability, bystander effect and delayed lethal mutation, are independent of p53 and occur through an epigenetic mechanism caused by oxygen stress (22, 23, 42–44).

Although AP-1 regulates cell survival, proliferation and apoptosis, the consequences of activating AP-1 are dependent on cell type and other environmental factors (37). While it may promote apoptosis in some cell types, AP-1 is required for the survival of others. For instance, inhibition of c-Jun activity by dominant negative mutants or neutralizing antibodies can protect neuronal cells from apoptosis (51, 52). Ectopic expression of c-Jun or c-Fos can induce apoptosis in some types of neurons and fibroblasts (53, 54). JNK activation, on the other hand, protects thymocytes from apoptosis induced by Fas stimulation (55). It is known that a tumor promoter, phorbol ester, prevents T-cell apoptosis induced by growth factor depletion (56). Inhibitors of AP-1 activation induce apoptosis of human leukemia cells (57). We also have reported that ascoclorin, an antibiotic that suppresses cellular AP-1 activity, selectively kills estrogen receptor (ER)-negative human and mouse breast cancer cell lines and prolongs the survival time of mice implanted with an ER-negative mammary carcinoma (58, 59). These observations demonstrate the homeostatic function of AP-1 (37), which reacts to changes in growth and environmental conditions to adjust the gene expression profile, allowing cells to adapt to continuous low-dose-rate γ-ray irradiation. It is noteworthy that the adaptive response induced by low-dose/low-dose-rate radiation is also dependent on a stress-related response (22, 23), although in our experiments the attenuation was not limited to DNA toxin-induced apoptosis but occurred in staurosporine-induced apoptosis (Fig. 4).

Correlation between AP-1 and malignancy in human breast cancers has been reported. Gene expression profiling of human breast cancer cells indicates that Fra-1 and vimentin, both of which are downstream targets of AP-1, are consistently associated with a highly aggressive phenotype (60). Overexpression of c-Jun in ER-positive cells increases Fra-1 expression and diminishes ER expression, resulting in increased aggressiveness (61). We also reported that ER-negative breast cancer cell lines have high AP-1 activity mainly through high Fra-1 constitutive expression (58, 59). In fact, AP-1 is a major regulator of matrix proteinases that play pivotal roles in tumor invasion and metastasis (62, 63). AP-1 also participates in various human diseases other than cancer, such as inflammatory diseases and metabolic diseases (64), which might in part explain the ameliorative effect of continuous low-dose-rate irradiation on diabetes and aging that we have observed (30–32).

Our results implicate apparently opposite outcomes in the context of radioprotection. A similar dose-rate-dependent growth retardation is observed in various cell lines, including normal human fibroblasts exposed to continuous low-dose-rate γ rays (35). Moreover, the results presented here suggest that growth retardation and apoptosis resistance are related to a stress-related response that would be intact in M059K cells and U2OS cells as well as normal human cells. Thus, chronic exposure to low-dose-rate radiation cytostatically attenuates normal cell growth in G1-phase cell cycle, increasing the radioresistance of normal tissues: a direct dose-rate effect. On the other hand, low-dose-rate radiation exposure increases c-Jun expression, leading to genetic instability and resistance to apoptosis evoked by chemotherapeutic agents, which increases the probability of oncogenesis and malignant tumor progression. It is crucial to precisely reevaluate the necessary dose rate, total dose, exposure time and dose fractionation for delivering effective radiation treatment while avoiding negative consequences such as induction of malignant tumor cell phenotypes. Our results also suggest that reevaluation of the irradiation schedule is important for risk estimation, protection and utilization of ionizing radiation for other therapeutic uses. This conclusion is based on two tumor cell lines that have impaired cell cycle checkpoints. Generalization to other cell lines, including normal human cell lines, as well as elucidation of the distinct mechanisms of radiation response, will be the subjects of future studies.

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