

Molecular response of human glioblastoma multiforme cells to ionizing radiation: cell cycle arrest, modulation of the expression of cyclin-dependent kinase inhibitors, and autophagy

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Object. Ionizing radiation is the gold-standard adjuvant treatment for glioblastoma multiforme (GBM), the most aggressive primary brain tumor. The mechanisms underlying neoplastic glial cell growth inhibition after administration of ionizing radiation, however, remain largely unknown. In this report, the authors characterize the response of GBM cells to ionizing radiation and elucidate factors that correlate with the radiosensitivity of these tumors.

Methods. Six human GBM cell lines were subjected to increasing doses of radiation. Each demonstrated a dose-dependent suppression of cell proliferation. In the most radiosensitive cell line, the authors demonstrated a transient increase in the expression of the cyclin-dependent kinase inhibitors (CDKIs) p21 and p27, which corresponded with a G₁ cell-cycle arrest. In contrast, the most radioresistant cell line demonstrated a decrease in p21 and p27 expression levels, which correlated with a failure to arrest. Apoptosis did not occur in any cell line following irradiation. Instead, autophagic cell changes were observed following administration of radiation, regardless of the relative radiosensitivity of the cell line.

Conclusions. These findings elucidate some of the molecular responses of GBMs to irradiation and suggest novel targets for future therapy.

KEY WORDS • glioblastoma multiforme • apoptosis • autophagy • ionizing radiation

GLIOMASTOMA multiforme is the most common primary brain tumor in adults. The median length of survival of patients with GBMs remains shorter than 1 year, despite concerted efforts to improve current therapies and develop new clinical approaches.²² Radiation therapy is the single most important adjuvant treatment that has consistently been shown to improve survival times in patients with GBMs.¹² Nevertheless, the cellular and molecular processes involved in the response of GBM cells to administration of ionizing radiation remain largely unknown.

When irradiated, many nonglial cancer cells undergo cell death by apoptosis,¹³ a mechanism of active cell death characterized by rapid loss of plasma membrane integrity, DNA fragmentation, and expression of numerous genes.⁶ Apoptosis is not the only potential cellular response to radiation, however. In addition to cell-cycle arrest, one can infer from the recent literature that an alternative response to irradiation involves the lysosomal compartment rather than the nucleus.¹⁷ This process, known as autophagy, is manifested by the formation of AVOs in the cytoplasm. The formation of AVOs does not always correlate with cell death. Instead,

AVO formation signifies the sequestration of intracellular toxins in the cytoplasm and, therefore, may represent a cellular defense mechanism.¹⁷

In the present study we report a novel response of human GBM cells to radiation treatment. Whereas glioma cells do not undergo apoptosis following irradiation, we demonstrate the formation and accumulation of AVOs in response to irradiation. This coincides with a G₁ cell-cycle arrest in radiosensitive cells and modulation of the expression of the CDKIs p21 and p27.

Materials and Methods

Cell Culture, Treatment With Ionizing Radiation, and Cell Viability Assay

The human GBM cell lines A172, GB-1, T98G, U251-MG, U373-MG, and U87-MG were cultured as adherent cells in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum, 100 µg/ml streptomycin, and 100 U/ml penicillin G. Tumor cells were seeded at 2×10^3 cells/well (0.1 ml) in 96-well flat-bottomed plates and incubated overnight at 37°C. Radiation treatment was given 24 hours postseeding (Time 0) at room temperature in a ¹³⁷Cs irradiator (Shepherd Mark-I [model SN1068]; J. L. Shepherd and Assoc., San Fernando, CA) at a dose rate of 5.1 Gy/minute. Control cells were transported to the irradiator, but not exposed to radiation. All cells were subsequently returned to the incubator.

To assess cell proliferation, trypsinized adherent cells were counted on Days 0 through 6 by trypan blue dye exclusion, as described previously.¹⁰ Briefly, the supernatant was removed and the cells were trypsinized (50 µl) for 10 minutes, after which an equal volume of

Abbreviations used in this paper: AVO = acidic vesicular organelle; CDK = cyclin-dependent kinase; CDKI = CDK inhibitor; FACS = fluorescence-activated cell sorting; GBM = glioblastoma multiforme; TUNEL = terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling.

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trypan blue dye (2%) was added and cell counts were performed using a hemocytometer. Each cell line was assayed at 0, 5, 10, and 20 Gy. Each assay was independently repeated five times for a total of six assays for each cell line at each dose of radiation.

Cell Cycle Analysis

Nonadherent and trypsinized adherent cells were pooled, stained with propidium iodide by using a commercially available kit (Cellular DNA Flow Cytometric Analysis Kit; Roche Molecular Biochemicals, Indianapolis, IN), and analyzed for DNA content by using a cytometer (FACScan flow cytometer; Becton Dickinson, San Jose, CA) at an excitation wavelength of 488 nm. The data were analyzed with the aid of a software program (Cell Quest; Becton Dickinson) with dead cells gated out using pulse processing. The A172 and T98G cells were tested without subjecting them to radiation treatment and on Days 1 through 4 following administration of radiation (5 Gy).

Analysis of Apoptosis and Autophagy

Apoptosis was assessed in all cell lines by performing TUNEL staining (ApopTag kit; Intergen Co., Purchase, NY) with a few minor modifications. Briefly, adherent cultured cells were washed with phosphate-buffered saline, fixed for 30 minutes in 1% paraformaldehyde in the saline solution, and rewashed. Apoptotic cells were enzymatically labeled with digoxigenin nucleotide by using terminal deoxynucleotidyl transferase. These cells were detected using peroxidase-conjugated anti-digoxigenin antibody enhanced with osmium tetroxide and were visualized by bright-field microscopy. The radiation doses that we tested were 0, 5, 10, 20, and 30 Gy on Days 2 and 4 following irradiation. Adenoviral p53-transfected cells were used as positive controls.

Quantitative analysis of apoptosis was achieved by performing a flow cytometric analysis of subdiploid DNA content in propidium iodide-stained cells with FACS analysis, as described earlier. Nonirradiated and irradiated (5 Gy) cells were tested on Days 1 through 4 following the radiation treatment.

Autophagy was assessed using supravital cell staining with acridine orange (Polysciences, Warrington, PA). Acridine orange was added to adherent unwashed cultured cells at a final concentration of 1 μ g/ml for a period of 20 minutes. Afterward the cells were trypsinized, pooled, and analyzed with the aid of a fluorescence microscope (Axioscop; Carl Zeiss, Inc., Thornwood, NY) equipped with a mercury 100-W lamp, a 490-nm band-pass blue excitation filter, a 500-nm dichroic mirror, and a 515-nm-long pass barrier filter. Microphotographs were digitally recorded by a computer. Acridine orange experiments were performed using A172 and T98G cells without the use of radiation and on Days 1 through 4 following radiation treatment (5 Gy).

Western Blot Assays With CDKs

Soluble protein for the immunoblot analysis was harvested from tumor cells lysed in 500 μ l of freshly prepared extraction buffer (10 mM Tris-HCl [pH 7], 140 mM sodium chloride, 3 mM magnesium chloride, 0.5% NP-40, 2 mM phenylmethylsulfonyl fluoride, 1% aprotinin, and 5 mM dithiothreitol) for 20 minutes on ice. Equal amounts of protein, as estimated by assay (Bio-Rad Protein Assay; Bio-Rad Laboratories, Richmond, CA) were separated by electrophoresis on a 10% polyacrylamide gel in sodium dodecyl sulfate and, thereafter, subjected to electrotransfer to nitrocellulose. The filters were subjected to immunoblot analysis by using the enhanced chemiluminescence detection system (Amersham, Arlington Heights, IL) according to the manufacturer's instructions. The specific antibodies that we used were those against p21 (Oncogene Science, Cambridge, MA) and p27 (Santa Cruz Biotechnology, Santa Cruz, CA). Equivalent sample loading of intact protein was confirmed by blotting while using anti-actin antibody (Santa Cruz Biotechnology). These experiments were performed using A172 and T98G cells exposed to either no radiation or 5-Gy radiation (Days 1 and 4). The band intensities were digitally quantified by using appropriate software (Un-Scan-It Gel; Silk Scientific Corp., Orem, UT). Each p21 and p27 band intensity was normalized to the correspond-

ing actin band to allow a quantitative comparison between protein samples.

Statistical Analysis

Cell proliferation data represent the average of six independent assays. Statistical proliferation differences were assessed using an unpaired Student *t*-test. A probability value less than 0.05 was considered significant.

Results

Effect of Ionizing Radiation on Human GBM Cell Proliferation

Escalating single doses of ionizing radiation (0, 5, 10, and 20 Gy) caused a dose-dependent decrease in cell proliferation in all six human GBM cell lines tested: A172, GB-1, T98G, U251-MG, and U373-MG, which contain mutant *p53*, and U87-MG, which contains wild-type *p53* (Fig. 1 *upper*). The assays revealed a significant inhibition of cell proliferation at 20 Gy for all cell lines within 24 hours after the radiation treatment. This inhibition persisted throughout the entire time course (6 days) following this treatment. At lower doses of radiation (5 and 10 Gy), the cell lines demonstrated different relative sensitivities. The cell lines that were relatively radiosensitive at these doses included A172 and GB-1. These cell lines demonstrated a significant suppression of cell proliferation following radiation treatment with 5 and 10 Gy within 24 hours after exposure. Relatively radioresistant cell lines included T98G, U251-MG, U373-MG, and U87-MG. These cell lines also demonstrated inhibition of cell proliferation, but only after they were subjected to higher radiation doses and evaluated at longer postirradiation intervals, compared with the radiosensitive cell lines. Inhibition of cellular proliferation in these radioresistant cell lines reached statistical significance after an exposure interval of 4 days following administration of 5 Gy and an interval of 2 days following administration of 10 Gy. The most radiosensitive cell line was A172, whereas the most radioresistant cell line was T98G, as demonstrated in Fig. 1 *lower*. To elucidate the molecular differences between radioresistance and radiosensitivity, we focused subsequent experiments on these two cell lines.

Growth Inhibition, Apoptosis, and Autophagy of Human GBMs in Response to Radiation Treatment

Nonirradiated A172 and T98G cells demonstrated equivalent cell-cycle distributions as determined by FACS analysis (Fig. 2). Following exposure to ionizing radiation, however, radiosensitive A172 and radioresistant T98G demonstrated different cell-cycle responses. Within 24 hours after a 5-Gy radiation treatment, A172 cells demonstrated cell-cycle arrest in the G_1 phase, with 92% of cells accumulating in the G_1 phase, compared with a baseline proportion of 76%. The cell-cycle distribution gradually returned to baseline within 4 days after radiation treatment. In contrast, T98G cells did not exhibit any perturbation of the cell cycle following exposure to the same dose of radiation.

To determine whether the suppression of GBM cell proliferation by ionizing radiation was attributable to apoptosis, TUNEL staining was performed. There was no positive TUNEL staining in irradiated A172 and T98G cells within 4 days after irradiation at all tested doses (Fig. 3). Fur-

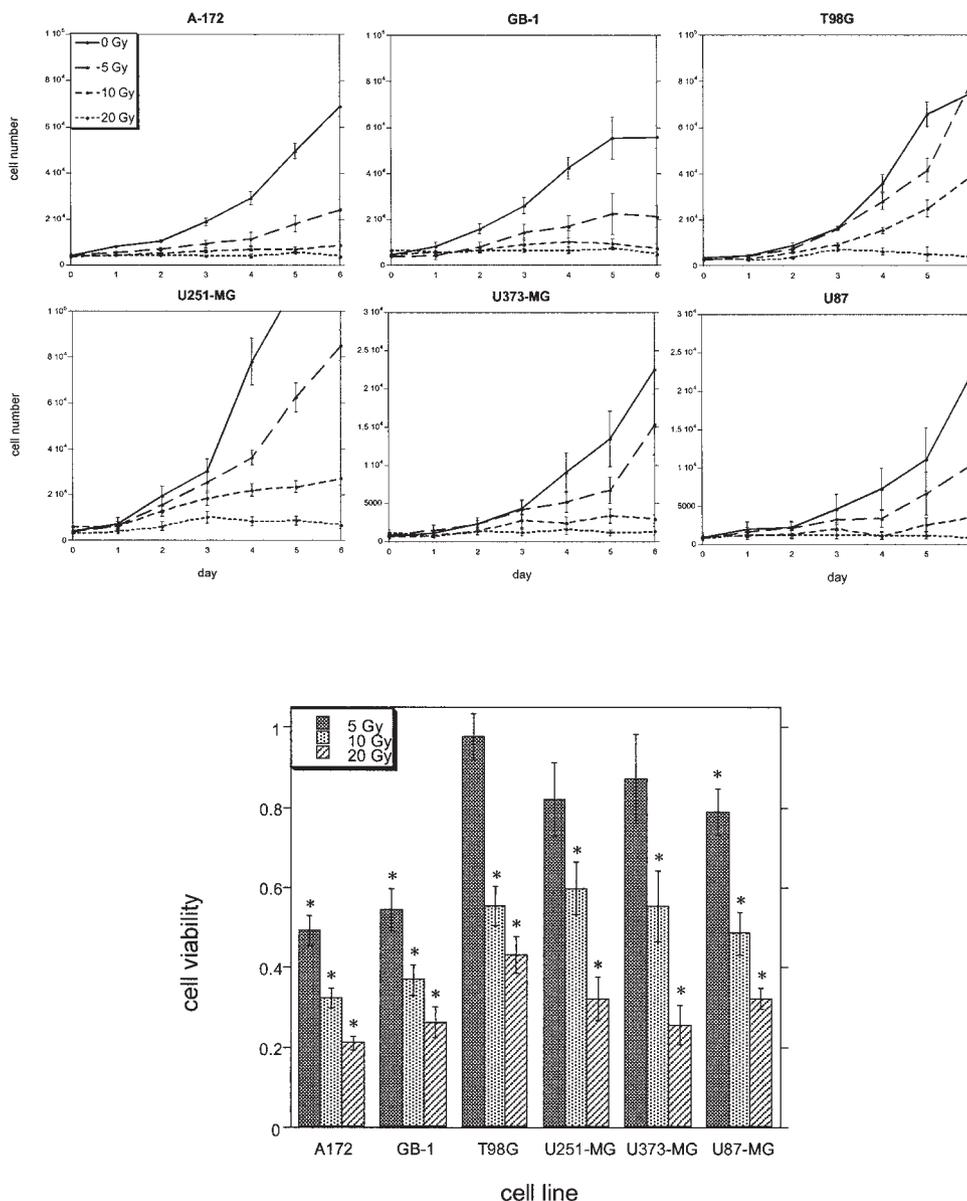


FIG. 1. Ionizing radiation effects on cell proliferation in six human GBM cell lines. *Upper:* Graphs showing that escalating single doses of ionizing radiation caused a dose-dependent inhibition of cell proliferation in each cell line. Bars represent standard deviations from the means. *Lower:* Bar graph demonstrating cell proliferation in radiated cell populations 3 days after radiation treatment compared with baseline nonirradiated cells. The A172 cell line exhibits the greatest radiosensitivity, whereas the T98G cell line displays the greatest resistance to radiation. *p < 0.05 compared with baseline values, unpaired Student t-test.

thermore, FACS analysis of irradiated A172 and T98G cells did not demonstrate accumulation of apoptotic sub-G₀ cells (Fig. 2).

To determine whether autophagy is a response of GBM cells to treatment with ionizing radiation, supravital staining with acridine orange was performed. Acridine orange accumulates in cytoplasmic AVOs, in which it forms aggregates that fluoresce. In both A172 and T98G cell lines, nonirradiated cells exhibited no cytoplasmic staining. In contrast, both cell lines demonstrated abundant cytoplasmic AVO formation in response to 5 Gy within 24 hours after irradiation and this persisted for 4 days following radiation treatment (Fig. 4).

Alterations in the Expression of CDKI in Response to Radiation Treatment

In the radiosensitive A172 cell line, the expression levels of the CDKIs p27 and p21 transiently increased within 1 day after exposure to radiation (5 Gy; Fig. 5). Band quantification demonstrated a 96% increase in p27 expression and an 86% increase in p21 expression relative to nonirradiated cells within 24 hours after radiation treatment. These protein levels gradually decreased to baseline expression levels during the next 3 days. In contrast, the radioresistant T98G cell line demonstrated an inverse, gradual, but sustained decrease in p27 and p21 expression levels. Within 4

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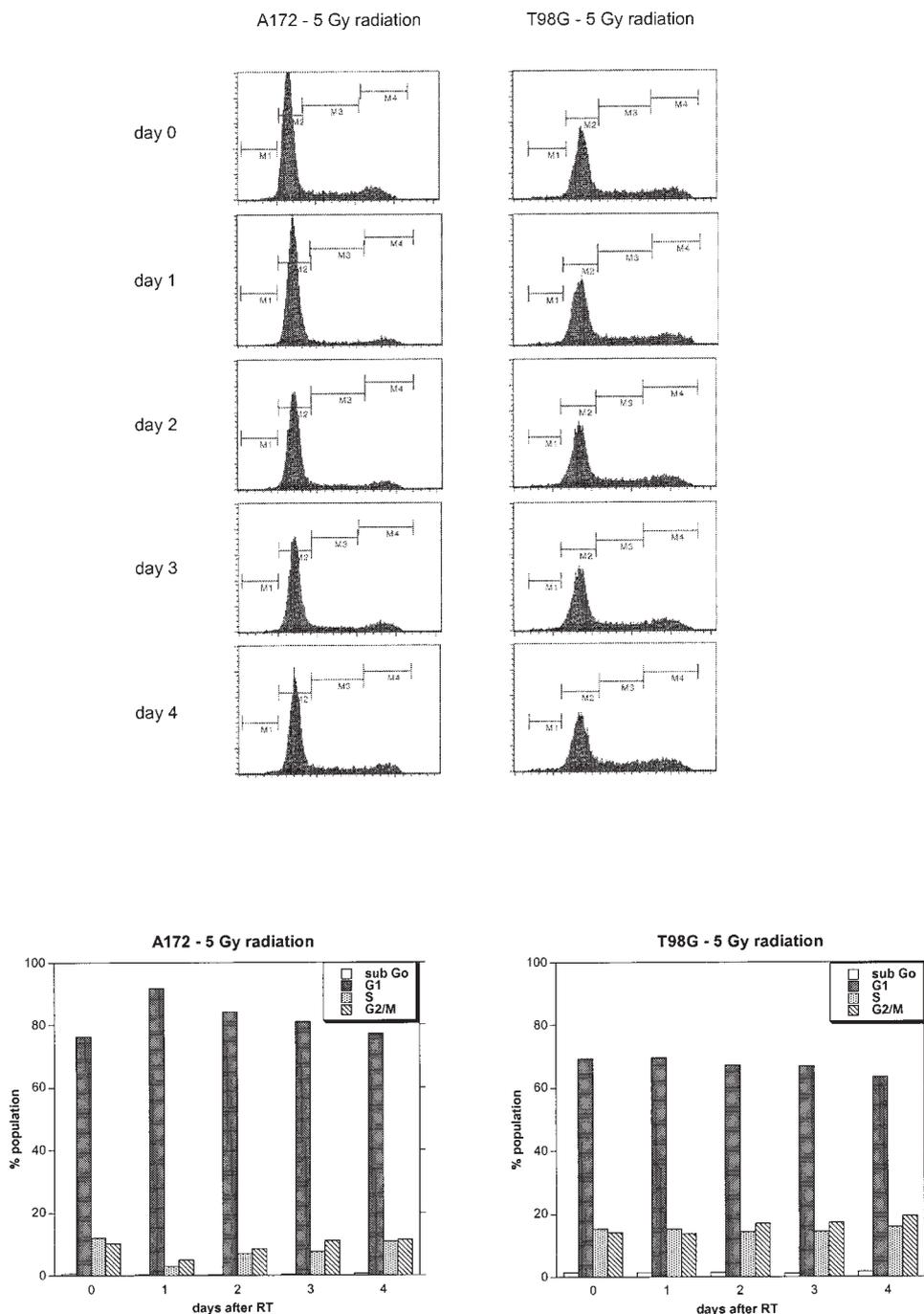


FIG. 2. Cell cycle distribution as assessed by FACS analysis. *Upper:* Histograms of DNA at baseline (Day 0) and at 1 through 4 days after exposure to 5-Gy radiation. Marker (M) 1 encompasses sub-G₀ cells; M2, G₁ cells; M3, S cells; and M4, G₂/M cells. *Lower:* Bar graphs demonstrating the percentage of cell population in each phase of the cell cycle. The A172 cells displayed a G₁ cell-cycle arrest within 1 day after radiation treatment; the number of cells gradually returned to baseline values within 4 days. The T98G cells did not exhibit any disruption in the cell-cycle distribution following radiation treatment. Neither cell line demonstrated an appreciable accumulation of apoptotic sub-G₀ cells.

days after irradiation, T98G cells demonstrated decreases in the expression level of p27 by 42% and in that of p21 by 33%.

Discussion

Ionizing radiation remains the most consistently used

therapy for patients with GBMs, although its clinical efficacy has been dismal. An understanding of the molecular responses of GBM cells to administration of radiation may offer potential new targets for future therapy. In this study we have assessed the relative radiosensitivities of six GBM cell lines. We found that in response to radiation treatment, all cell lines demonstrated a dose-dependent inhibition of

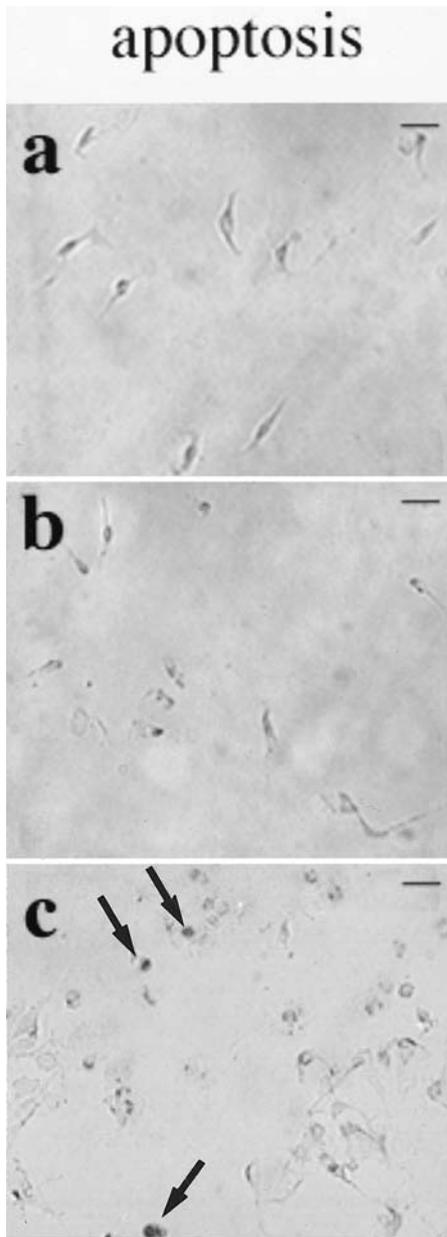


FIG. 3. Photomicrographs showing TUNEL staining of A172 cells not subjected to radiation therapy (a) and those examined 4 days after radiation treatment with 30 Gy (b). No TUNEL-positive apoptotic cells were observed. The U87-MG cells transfected with adenoviral *p53* served as a positive control (c) for apoptotic cells (arrows). Similar results were obtained with T98G cells (data not shown).

cell proliferation. Preliminary findings with extended assays conducted for 3 weeks (data not shown) have indicated that resumption of cellular proliferation occurs even in the most radiosensitive cell line (A172) following exposure to the highest radiation dose tested (20 Gy). These results correlate with those from clinical studies in patients with GBMs who have undergone radiation therapy, which demonstrate a transient inhibition of tumor growth followed by regrowth within 6 to 8 months after irradiation of the lesion.

To elucidate factors crucial to the radiosensitivity of

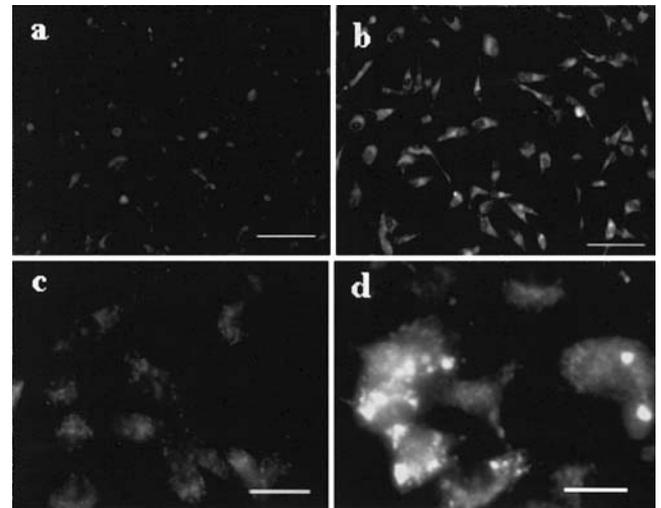


FIG. 4. Immunofluorescence photomicrographs after supravital staining with acridine orange for detection of AVOs. a and c: Nonirradiated A172 cells demonstrating minimal presence of AVOs. b and d: Irradiated A172 cells 1 day after exposure to 5 Gy displaying abundant cytoplasmic AVO formation. Similar results were obtained with T98G cells (data not shown). Bars = 100 μm in a and b, and 20 μm in c and d.

GBM cells, we compared the molecular responses of the most radiosensitive cell line (A172) and the most radioresistant cell line (T98G). Similar to other previous studies, we found that radiation did not induce apoptosis in any of the GBM cell lines tested in this study, regardless of the *p53* status.^{11,24} Apoptosis, or “programmed cell death,” has been shown to occur with a degree of heterogeneity in different tumors.¹⁴ In particular, apoptosis of glioma cells has only been reported in a few studies.^{3,23} Enhancement of apoptosis after irradiation of malignant glioma cells has been the focus of tremendous research efforts in neurooncology. Several strategies have been reported to accomplish this goal, including exogenous transfer of *p53*, *APAF-1*, and *caspase*. Gomez-Manzano, et al.,⁷ demonstrated that glioma cells containing mutant *p53* undergo apoptosis after exogenous *p53* transfection by adenovirus, whereas wild-type *p53* cells do not. Lang, et al.,¹¹ showed enhancement in the radiosensitivity of wild-type *p53* human glioma cells by adenovirus-mediated delivery of *p53*. Subsequently, his group elegantly demonstrated that induction of apoptosis after adenovirus-*p53* transfer is dependent on site-specific phosphorylation at serine sites 15 and 20.²⁰ Shinoura, et al.,¹⁹ showed that transduction of U373-MG cells with *caspase 9* by adenovirus resulted in increased radiation-induced apoptosis.

Apoptosis, however, is not the sole response to cellular damage. Autophagic cell death or “type II programmed cell death,” recently has been described as an alternative response to cellular damage.^{4,5} Activated by environmental factors, autophagy is a degradative pathway characterized by the formation of membrane-bound vacuoles in the cytoplasm.⁴ These autophagic vacuoles, otherwise known as AVOs, sequester cytosolic components and have been implicated in cellular defense mechanisms such as protection against infectious agents,²¹ drug resistance,² and survival during starvation conditions.¹⁵ The continued accumulation

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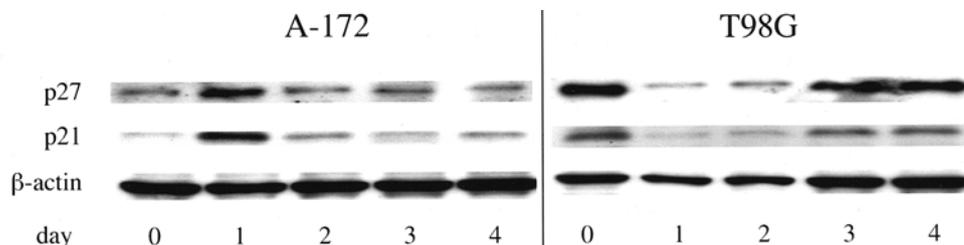


FIG. 5. Results of the Western blot assay of CDKIs. The A172 cells demonstrate a transient increase in p27 and p21 expression within 24 hours after exposure to 5 Gy, with a return to baseline by Day 4. The T98G cells demonstrate an inverse, sustained decrease in p27 and p21 expression within 24 hours after irradiation.

of AVOs after high levels of damage may negate their protective effect, however, leading to eventual autophagocytosis and cell death.¹⁷ It is noteworthy that ionizing radiation treatment of tumor cell lines unrelated to the brain has also failed to induce apoptosis. In addition, death-inducing agents other than ionizing radiation have been reported to produce cell death without apoptosis.⁵ Cells that survive administration of radiation may continue to divide and form colonies. Although we did not test the fate of irradiated cells in this study, it has been reported that, in cell lines from other organs, the progenies of irradiated cells contain an increased level of AVOs.¹⁷ This suggests that the presence of AVOs may protect the cell against radiation-induced damage or lead to cell death. The presence of AVOs may protect the cell by preventing cytoplasm acidification, by providing catabolites required for repair process, and/or by containing toxic molecules. Nevertheless, continuous accretion of AVOs might offset their protective effects and result in cell death. In our study, we demonstrated the presence of AVOs in both radioresistant and radiosensitive GBM cell lines. Thus, we postulate that an additional step after AVO formation is necessary to lead to the cell's demise.

The regulation of the cell cycle is dependent on various check points.¹⁶ At the critical restriction check point in the late G₁ phase, the cell is committed to enter into the S phase and no longer requires mitogenic growth factors to complete cell division. With the discovery of CDK came the understanding of how progression through the G₁ phase of the cell cycle may be positively regulated. On the other hand, the modulation of various CDKIs, such as p27 and p16, can cause a lack of progression through the G₁ phase, leading to G₁ arrest.¹ Protein 27 regulates the G₁-S transition of the cell cycle by inhibiting cyclins D, E, and A. The expression of p27 is thought to be regulated by posttranslational degradatory pathways.¹⁸ In our experiments, the human GBM line sensitive to ionizing radiation showed a transient increase in the CDKIs p27 and p21 within 24 hours after exposure to the radiation. This corresponded to a cell-cycle arrest in the G₁ phase, as demonstrated by FACS analysis. On the contrary, the p27 and p21 levels decreased within 24 hours after administration of radiation and remained suppressed in the radioresistant cell line. Several studies have shown that p53-dependent G₁ arrest is mediated through the induction of p21 and the resulting inhibition of CDK.^{8,9} In our study, however, the radioresistant and radiosensitive cell lines both have mutant p53 gene status. Thus, the G₁ arrest observed in this study might be independent of an effect of p53. The causative effects of

the increased expression of p27 and p21 on radiosensitivity cannot be fully elucidated by the findings of our study.

Conclusions

We have demonstrated the occurrence of autophagy in human GBM cells after exposure to ionizing radiation. We have shown a difference in cell-cycle arrest and expression of CDKI when radiosensitive and radioresistant human GBM cell lines are compared. Although the findings of this study cannot provide a full mechanistic explanation to correlate these phenomena, we suggest that modulation of CDKI inhibitors should be further investigated as a potential novel target to increase the sensitivity of human glioma cells to radiation.

References

- Alleyne CH Jr, He J, Yang J, et al: Analysis of cyclin dependent kinase inhibitors in malignant astrocytomas. *Int J Oncol* **14**: 1111-1116, 1999
- Altan N, Chen Y, Schindler M, et al: Defective acidification in human breast tumor cells and implications for chemotherapy. *J Exp Med* **187**:1583-1598, 1998
- Bauman GS, Fisher BJ, McDonald W, et al: Effects of radiation on a three-dimensional model of malignant glioma invasion. *Int J Dev Neurosci* **17**:643-651, 1999
- Bursch W: The autophagosomal-lysosomal compartment in programmed cell death. *Cell Death Differ* **8**:569-581, 2001
- Bursch W, Ellinger A, Kienzl H, et al: Active cell death induced by the anti-estrogens tamoxifen and ICI 164 384 in human mammary carcinoma cells (MCF-7) in culture: the role of autophagy. *Carcinogenesis* **17**:1595-1607, 1996
- Estus S, Zaks WJ, Freeman RS, et al: Altered gene expression in neurons during programmed cell death: identification of c-jun as necessary for neuronal apoptosis. *J Cell Biol* **127**:1717-1727, 1994
- Gomez-Manzano C, Fueyo J, Kyritsis AP, et al: Adenovirus-mediated transfer of the p53 gene produces rapid and generalized death of human glioma cells via apoptosis. *Cancer Res* **56**: 694-699, 1996
- Haas-Kogan DA, Yount G, Haas M, et al: p53-dependent G1 arrest and p53-independent apoptosis influence the radiobiologic response of glioblastoma. *Int J Radiat Oncol Biol Phys* **36**: 95-103, 1996
- Harper JW, Adami GR, Wei N, et al: The p21 Cdk-interacting protein Cip 1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* **75**:805-816, 1993
- Kondo S, Kondo Y, Li G, et al: Targeted therapy of human malignant glioma in a mouse model by 2-5A antisense directed against telomerase RNA. *Oncogene* **16**:3323-3330, 1998

11. Lang FF, Yung WK, Raju U, et al: Enhancement of radiosensitivity of wild-type p53 human glioma cells by adenovirus-mediated delivery of the p53 gene. **J Neurosurg** **89**:125–132, 1998
12. Leibel SA, Sheline GE: Radiation therapy for neoplasms of the brain. **J Neurosurg** **66**:1–22, 1987
13. Lowe SW, Ruley HE, Jacks T, et al: p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. **Cell** **74**:957–967, 1993
14. Meyn RE, Stephens LC, Ang KK, et al: Heterogeneity in the development of apoptosis in irradiated murine tumors of different histologies. **Int J Radiat Biol** **64**:583–591, 1993
15. Mizushima N, Noda T, Yoshimori T, et al: A protein conjugation system essential for autophagy. **Nature** **395**:395–398, 1998
16. Nurse P: Ordering S phase and M phase in the cell cycle. **Cell** **79**:547–550, 1994
17. Paglin S, Hollister T, Delohery T, et al: A novel response of cancer cells to radiation involves autophagy and formation of acidic vesicles. **Cancer Res** **61**:439–444, 2001
18. Piva R, Cancelli I, Cavalla P, et al: Proteasome-dependent degradation of p27/kip1 in gliomas. **J Neuropathol Exp Neurol** **58**:691–696, 1999
19. Shinoura N, Sakurai S, Asai A, et al: Over-expression of APAF-1 and caspase-9 augments radiation-induced apoptosis in U-373MG glioma cells. **Int J Cancer** **93**:252–261, 2001
20. Shono T, Tofilon PJ, Schaefer TS, et al: Apoptosis induced by adenovirus-mediated p53 gene transfer in human glioma correlates with site-specific phosphorylation. **Cancer Res** **62**:1069–1076, 2002
21. Steinman RM, Moberg CL: Zanvil Alexander Cohn 1926–1993. **J Exp Med** **179**:1–30, 1994
22. Valtonen S, Timonen U, Toivanen P, et al: Interstitial chemotherapy with carmustine-loaded polymers for high-grade gliomas: a randomized double-blind study. **Neurosurgery** **41**:44–49, 1997
23. Yount GL, Afshar G, Ries S, et al: Transcriptional activation of TRADD mediates p53-independent radiation-induced apoptosis of glioma cells. **Oncogene** **20**:2826–2835, 2001
24. Yount GL, Haas-Kogan DA, Vidair CA, et al: Cell cycle synchrony unmasks the influence of p53 function on radiosensitivity of human glioblastoma cells. **Cancer Res** **56**:500–506, 1996

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