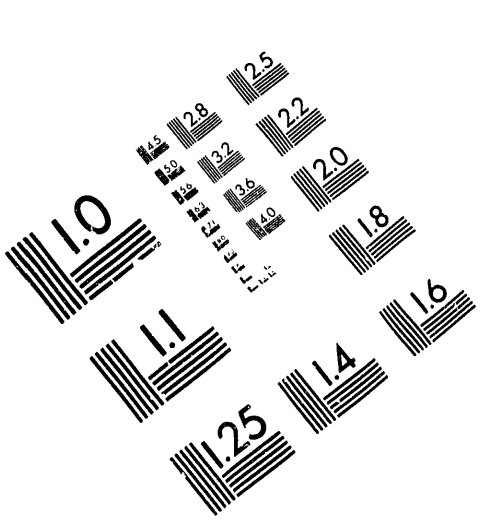
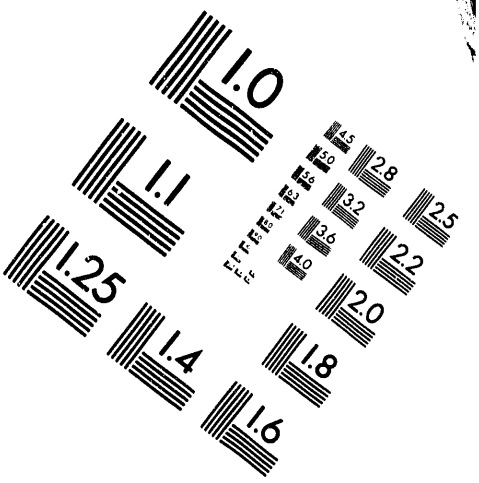
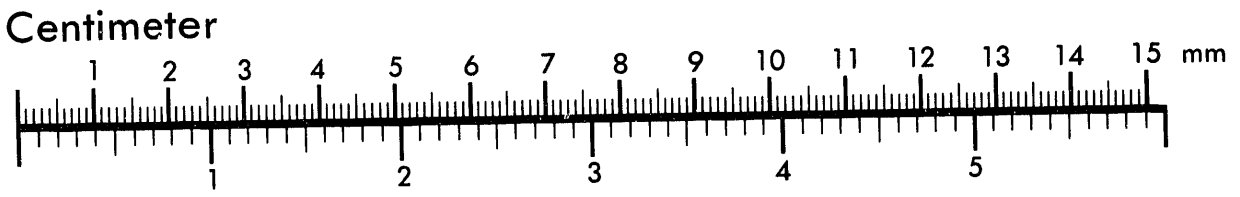
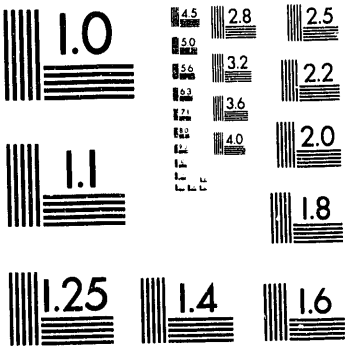
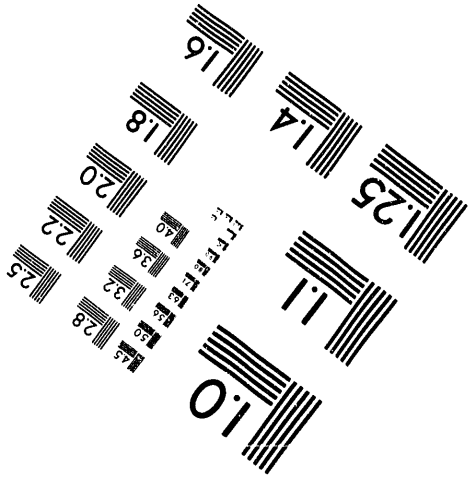


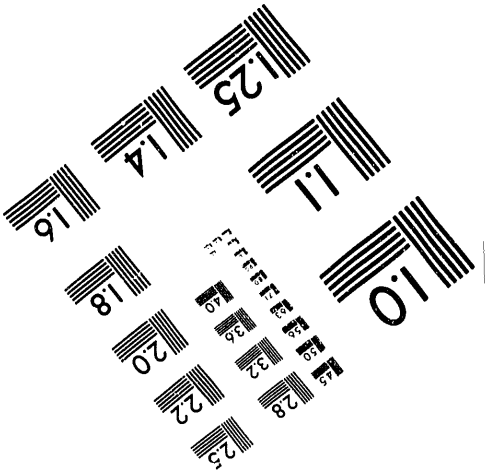
Association for Information and Image Management
1100 Wayne Avenue, Suite 1100
Silver Spring, Maryland 20910
301/587-8202











MANUFACTURED TO AIIM STANDARDS
BY APPLIED IMAGE, INC.

1 of 1

Conf-9305202--2

Radiation Research

Conference: Molecular, Genetic and Cellular Basis of Radiosensitivity at Low Doses: A Case of Induced Repair?
May 9–13, 1993, Vancouver, British Columbia, Canada

THE ROLE OF CONSTITUTIVE AND INDUCIBLE PROCESSES IN THE RESPONSE OF HUMAN SQUAMOUS CELL CARCINOMA CELL LINES TO IONIZING RADIATION[1]

JEFFREY L. SCHWARTZ

Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois 60439-4833; and
Department of Radiation and Cellular Oncology and the Cancer Research Center, The University of Chicago, Chicago, Illinois 60637

[1]This work was supported by the U.S. Department of Energy under contract No. W-31-109-ENG-38 and grant No. DE-FG02-88ER60661; by grant CA 42596 from the NCI, The Center for Radiation Therapy; and a Faculty Research Award from the American Cancer Society.

Person to whom all correspondence should be sent:

Jeffrey L. Schwartz
Center for Mechanistic Biology and Biotechnology
Argonne National Laboratory
9700 South Cass Avenue
Argonne, Illinois 60439-4833
Telephone: (708) 252-3919
Facsimile: (708) 252-3387

Schwartz, J. L. The Role of Constitutive and Inducible Processes in the Response of Human Squamous Cell Carcinoma Cell Lines to Ionizing Radiation. *Radiat. Res.*

The inherent radiation sensitivity of the cells within a tumor is thought to contribute to the success or failure of radiation therapy. In vitro studies have shown that radiation sensitivity differences in squamous cell carcinoma cell lines reflect alterations in DNA repair. These alterations result from constitutive changes in chromosome organization, not radiation-inducible processes. While inducible responses may play some role in the radiation response of tumor cells, there is no evidence for their involvement in inherent tumor cell radiosensitivity differences or in the success or failure of radiotherapy for squamous cell carcinomas.

The observation that radiation therapy selects for tumors that contain more radioresistant cells has suggested that the inherent sensitivity of the cells within a tumor plays a major role in the success or failure of radiation therapy (*1–3*). Recent reports on radiation-induced activation of transcription factors and their subsequent effect on gene expression (*4–6*) suggest that the radioresistance phenotype might be the result of radiation-inducible processes. Radiation-induced alterations in gene expression could influence radiation sensitivity through effects on cell cycle progression, apoptosis, or DNA repair. A review of the in vitro characteristics of radioresistant and radiosensitive tumor cell lines might provide clues as to the relative role of constitutive and inducible processes in the response of human tumor cells to radiation. To address the question of whether any of these radiation-induced alterations in gene expression underlie the radiation sensitivity of human tumor cells, a group of eight squamous cell carcinoma cell lines have been extensively characterized in this laboratory. Four of these cell lines are relatively radiosensitive, having D_0 values between 1.0 Gy and 1.6 Gy. The other four cell lines are relatively radioresistant with D_0 values between 2.2 Gy and 3.5 Gy (Figure 1).

Cell Cycle Progression Following Radiation Exposure

One likely consequence of radiation-induced alterations in gene expression is the production of transient blocks in G_2, at the G_1/S border, and in S phase. Alterations in the control of these cell cycle checkpoints have been implicated in radiation sensitivity. For example, the radioresistance that is seen in oncogene-transformed cell lines is associated with an extended G_2 delay following radiation exposure (*7,8*). The analysis of G_2 delay in two radioresistant and two radiosensitive squamous cell carcinoma cell lines following a 5-Gy exposure to gamma rays is shown in Figure 2. A prolonged G_2 was found for one of the

resistant and one of the sensitive cell lines. A short G_2 was seen in the other two cell lines. For squamous cell carcinoma cell lines, therefore, there is no simple relationship between G_2 delay and radiation sensitivity.

The p53 gene product has been shown to be an important component of the radiation-induced G_1/S block (*9*). While the presence or absence of a normal p53 gene product does influence cell cycle progression in the tumor cell lines, it has no apparent effect on radiation sensitivity. Five of the cell lines analyzed (three resistant and two sensitive) showed p53 alterations. Three of the cell lines, including the most resistant and the most sensitive lines, showed no p53 alterations (*10*, Brachman, personal communication).

Similarly, no relationship was found between the sensitivity of these cells to the cytotoxic effects of x-rays and their sensitivity to radiation-induced DNA synthesis inhibition (*11*). Only one of the eight cell lines showed a radioresistant DNA synthesis, while the other seven lines were relatively sensitive to DNA synthesis inhibition. Therefore, the different tumor cell line radiation sensitivities are not due to any radiation-induced alterations in cell growth kinetics.

Apoptosis

Cell killing in tumor cells likely involves both mitotic and non-mitotic or apoptotic processes. As gene expression has been linked to apoptosis, one effect of radiation-induced alterations in gene expression could be to modulate apoptosis. For the squamous cell carcinoma cell lines, however, the dominant form of cytotoxicity results from mitotic processes. There is no evidence for the characteristic DNA fragmentation seen with apoptosis following radiation exposure, and there is a linear relationship between cell killing and induced chromosome aberration frequency (*12*), as one would expect for mitotic cell death.

DNA Repair Characteristics

Extensive evidence from this laboratory and others suggests that DNA repair alterations underlie the radiation sensitivity of tumor cell lines. DNA double-strand break rejoining analysis by neutral filter elution (*13–15*) and pulse-field gel electrophoresis (*16*), as well as chromosome break rejoining analysis (*14*), all suggest that the kinetics of rejoining are faster in radioresistant cell lines. Analysis of the types of chromosome aberrations induced (*14,12*) suggest that the faster rejoining leads to greater fidelity in repair as x-rays induce fewer chromosome exchange-type aberrations in resistant cell lines.

The alterations in DNA repair are closely associated with alterations in chromosome organization. The DNA in chromosomes is organized on many different levels (*17*). At one level of organization, the DNA exists as a supercoiled loop attached to a protein structure, the DNA-nuclear matrix. When breaks are induced in this supercoiled loop, the loop relaxes and unwinds. This can be detected by DNA neutral filter elution, alkaline unwinding, and nucleoid-based assays of DNA damage (*18,19*). In the radioresistant tumor cell lines, there is an apparent constraint to unwinding following radiation exposure. Limiting DNA unwinding may facilitate the rejoining of DNA strand breaks by keeping broken ends in close proximity for a more rapid and accurate rejoining.

These alterations in DNA repair and chromosome organization are most likely the result of constitutive, and not inducible, processes. All of the DNA unwinding assays which show correlations between chromosome organization and DNA repair involve the analysis of unirradiated cells or cells that are irradiated on ice and then immediately assayed. These alterations are by definition constitutive changes, not inducible ones. In addition, the inhibition of radiation-induced gene activation by protein kinase inhibitors such as

staurosporin or sangivamycin has no effect on the ability of cells to repair radiation-induced DNA breaks (*20*).

The Role of Inducible and Constitutive Processes in Tumor Response to Radiotherapy

The in vitro data on the squamous cell carcinoma cell lines suggest that radiation sensitivity differences in these cells result from constitutive changes in chromosome organization which influence the repair of DNA double-strand breaks. The results suggest that inducible responses play no major role in the inherent radiation sensitivity differences seen in squamous cell carcinoma cell lines. There is, therefore, no evidence for the involvement of inducible responses in the success or failure of radiotherapy for squamous cell carcinomas. The results do not suggest that inducible responses play no role in the radiation response of tumor cells. Inhibition of protein kinase activity will sensitize these squamous cell carcinoma cell lines to the cytotoxic effects of x-rays (*20*). Inducible responses probably play an important role in the radiotherapy response for other tumor types, such as lymphoid tumors, or other types of therapies, such as low-dose-rate therapies, where apoptosis is likely to be important. For squamous cell carcinoma cell lines, however, the inherent radiosensitivity differences observed reflect constitutive and not inducible processes.

REFERENCES

1. R. R. Weichselbaum, M. A. Beckett, J. L. Schwartz, and A. Dritschilo, Radioresistant tumor cells are present in head and neck carcinomas that recur after radiotherapy. *Intl. J. Radiat. Oncol. Biol. Phys.* **15,** 575-579 (1988).

2. R. R. Weichselbaum, M. A. Beckett, and J. L. Schwartz, Where do radioresistant cells come from? In *Radiation Research: A Twentieth-Century Perspective* (W. C. Dewey, M. Edington, R. J. M. Fry, E. J. Hall, and G. F. Whitmore, Eds.), Vol. II: Congress. Proc., pp. 751–755. Academic Press, San Diego, 1992.

3. J. L. Schwartz, M. A. Beckett, R. Mustafi, A. T. M. Vaughan, and R. R. Weichselbaum, Evaluation of different in vitro assays of inherent sensitivity as predictors of radiotherapy response. In *Radiation Research: A Twentieth-Century Perspective* (W. C. Dewey, M. Edington, R. J. M. Fry, E. J. Hall, and G. F. Whitmore, Eds.), Vol. II: Congress. Proc., pp. 716–721. Academic Press, San Diego, 1992.

4. G. Woloschak, C. Chang-Liu, and P. Shearin-Jones, Regulation of protein kinase C by ionizing radiation. *Cancer Res.* **50,** 3963–3967 (1990).

5. D. E. Hallahan, V. P. Sukhatme, M. L. Sherman, S. Virudachalam, D. W. Kufe, and R. R. Weichselbaum, Protein kinase-C mediates x-ray inducibility of nuclear signal transducers, egr-1 and c-jun. *Proc. Natl. Acad. Sci. USA* **88,** 2152–2160 (1991).

6. D. E. Hallahan, S. Virudachalam, M. L. Sherman, D. W. Kufe, and R. R. Weichselbaum, Tumor necrosis factor gene expression is mediated by protein kinase C following activation by ionizing radiation. *Cancer Res.* **51,** 4565–4569 (1991).

7. W. G. McKenna, G. Iliakis, M. C. Weiss, and R. J. Muschel, Increased G2 delay in radiation-resistant cells obtained by transformation of primary rat embryo cells with the oncogenes H-*ras* and v-*myc*. *Radiat. Res.* **125,** 283–287 (1991).

8. L.-N. Su and J. B. Little, Prolonged cell cycle delay in radioresistant human cell lines transfected with activated *ras* oncogene and/or Simian virus 40 T-antigen. *Radiat. Res.* **133,** 73–79 (1993).

9. M. B. Kasten, O. Inyekwere, D. Sidransky, B. Vogelstein, and R. W. Craig, Participation of *p53* protein in the cellular response to DNA damage. *Cancer Res.* **51,** 6304–6311 (1991).

10. D. G. Brachman, D. Graves, E. Vokes, M. Beckett, D. Haraf, A. Montag, E. Dunphy, R. Mick, D. Yandell, and R. R. Weichselbaum, Occurrence of *p53* gene deletions and human papilloma virus infection in human head and neck cancers. *Cancer Res.* **52,** 4832–4836 (1992).

11. A. Chen and J. L. Schwartz, Inhibition and recovery of DNA synthesis in human tumor cell lines following radiation exposure. *Radiat. Res.* **118,** 375–380 (1989).

12. J. L. Schwartz, The radiosensitivity of the chromosomes of the cells of human squamous cell carcinoma cells. *Radiat. Res.* **129,** 96–101 (1992).

13. J. L. Schwartz, M.B. Cohen, J. Rotmensch, S. M. Giovanazzi, and R. R. Weichselbaum, Faster repair of DNA double-strand breaks in radioresistant human tumor cells. *Intl. J. Radiat. Oncol. Biol. Phys.* **15,** 907-912 (1988).

14. J. L. Schwartz and A. T. M. Vaughan, Association between DNA/chromosome break rejoining rates, chromatin structure alterations and radiation sensitivity in human tumor cell lines. *Cancer Res.* **49,** 5054–5057 (1989).

15. J. L. Schwartz, R. Mustafi, M. A. Beckett and R. R. Weichselbaum, Prediction of human squamous cell carcinoma cell line radiation sensitivity by DNA filter elution measurements. *Radiat. Res.* **123,** 1–6 (1990).

16. A. J. Giaccia, J. L. Schwartz, J. Shieh, and J. M. Brown, The use of asymmetric-field inversion gel electrophoresis to predict tumor cell radiosensitivity. *Radio. Oncol.* **24,** 231–238 (1992).

17. W. G. Nelson, K. J. Pienta, E. R. Barrack, and D. S. Coffey, The role of the nuclear matrix in the organization and function of DNA. *Ann. Rev. Biophys. Chem.* **15,** 57–475 (1986).

18. P. L. Olive, DNA organization affects cellular radiosensitivity and detection of initial DNA strand breaks. *Intl. J. Radiat. Biol.* 62, 389–396 (1992).

19. J. L. Schwartz and A. T. M. Vaughan, DNA-nuclear matrix interactions and ionizing radiation sensitivity. *Environ. Molec. Mutagen.* (in press).

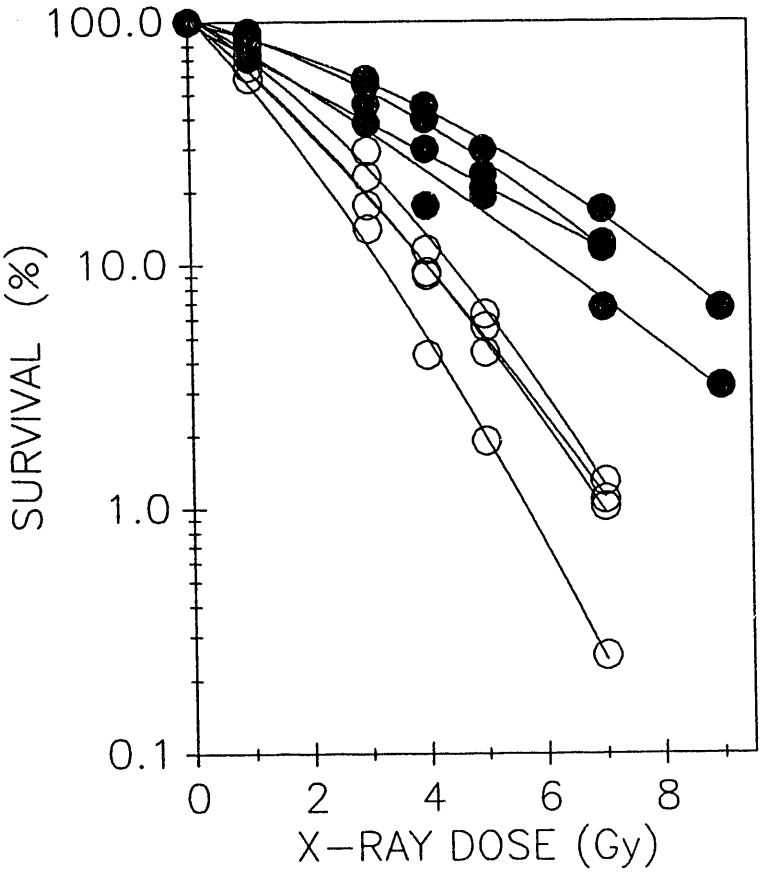
20. D. E. Hallahan, S. Virudachalam, J. L. Schwartz, N. Panje, R. Mustafi, and R. R. Weichselbaum, Inhibition of protein kinases sensitizes human tumor cells to ionizing radiation. *Radiat. Res.* **129,** 345–350 (1992).

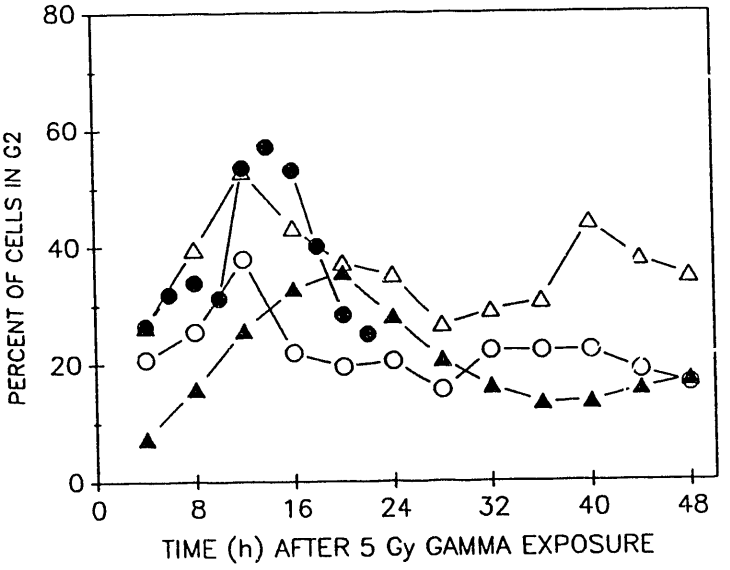
21. J. L. Schwartz, R. Mustafi, M. A. Beckett, E. A. Czyzewski, E. Farhangi, D. J. Grdina, J. Rotmensch, and R. R. Weichselbaum, Radiation-induced DNA double-strand break frequencies in human squamous cell carcinoma cell lines of different radiation sensitivities. *Intl. J. Radiat. Biol.* **59:** 1341–1352 (1991).

FIGURE LEGENDS

Figure 1. X-ray survival of squamous cell carcinoma cell lines. Filled symbols: radioresistant cell lines. Open symbols: radiosensitive cell lines.

Figure 2. G_2 delay induced by 5 Gy of gamma rays in radioresistant (filled symbols) and radiosensitive (open symbols) cell lines. The proportion of cells in G_2 was determined as described previously (*21*).


100.0
10.0
1.0
0.1
SURVIVAL (%)
0
2
4
6
8
X-RAY DOSE (Gy)


80
60
40
20
0
PERCENT OF CELLS IN G2
0
8
16
24
32
40
48
TIME (h) AFTER 5 Gy GAMMA EXPOSURE

DATE
FILMED
8/18/93
END