RADIOSENSITIVITY AND RAS ONCOGENE EXPRESSION IN PRENEOPLASTIC RAT TRACHEAL EPITHELIAL CELLS

PRINCIPAL INVESTIGATORS
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Abstract — The sensitivity of preneoplastic rat tracheal epithelial (RTE) cells to the cytotoxic effects of high- and low-LET radiation, and the modulating effect of the viral ras oncogene on this sensitivity were determined. Two lines of preneoplastic RTE cells have the same responsiveness to high-LET radiation, but differ in their responsiveness to a transfected ras oncogene and in their sensitivities to low-LET radiation. Cells that respond to ras by becoming neoplastic are more resistant to the cytotoxic effects of low-LET radiation than cells that are not transformable by ras. The radiosensitivity of ras-responsive cells was not altered by transfection with ras. However, transfection of ras-nonresponsive cells with ras decreased their sensitivity to low-LET radiation. These data suggest that the ability of cells to repair radiation damage changes as they progress to neoplasia.

Defining cellular responses to radiation-induced damage and identifying factors that modify those responses are critical aspects in developing models for the role of radiation in carcinogenesis. A recent report[1] describes a decrease in the radiosensitivity of NIH3T3 cells following transfection with a mutationally activated ras oncogene. These results suggest that the ras oncogene may directly or indirectly regulate repair or other cellular processes leading to an altered responsiveness to radiation.

Two preneoplastic variants of rat tracheal epithelial (RTE) cells that differ in their responsiveness to ras-induced neoplastic transformation have been identified (this report, pp. 370-373). These variants are presumed to be at different stages or on different pathways of progression to neoplasia. The purpose of the studies described here is to characterize the sensitivity of a ras-responsive and a ras-nonresponsive variant to high- and low-LET radiation, and to determine if transfection with a viral ras oncogene alters the radiosensitivity of these cells.

METHODS

Preneoplastic RTE cell lines, EGV 1 and EGV 9, previously isolated following exposure of normal RTE cells to the carcinogen N-methyl-N'-nitro-N-nitrosoguanidine,[2] were used in this study. Cells containing the viral Harvey ras oncogene were isolated following transfection of each cell line with ras DNA as previously described.[3] Prior to transfection with ras, both cell lines are nontumorigenic when injected subcutaneously into athymic nude mice. EGV 9, but not EGV 1, cells become tumorigenic following transfection with ras. The cytotoxic response of cells to radiation was determined in colony-forming assays. Cells were plated in plastic culture dishes for exposure to X rays, or in specially constructed culture dishes with a 1.5 μm Mylar film serving as the bottom for α-particle exposures. Approximately 20 h after plating, cells were exposed to X rays at a dose rate of approximately 300 mGy/minute, using a Picker X-ray therapy machine. Cells were exposed to α-particles, at a dose rate calculated to be approximately 2.1 mGy/sec, by placing cultures on electroplated sources of plutonium-238. After 7 days cultures

were fixed with ethanol and stained with Giemsa. Colonies with ≥ 30 cells were scored. The relative colony-forming efficiency, an index of cell killing, was calculated as the colony-forming efficiency of treated groups divided by the colony-forming efficiency of untreated controls.

RESULTS

Preneoplastic RTE cell lines, EGV 1 and EGV 9, showed similar exponential dose-dependent cytotoxic responses to α-particles (Fig. 1). Transfection of either cell line with the *ras* oncogene did not alter its responsiveness to high-LET radiation (Fig. 1).

In contrast, these cell lines differed in their sensitivities to X rays and in the effect of the *ras* oncogene on their response. EGV 1 originally showed an exponential decrease in cell survival following exposure to X rays, while the dose response curve for EGV 9 had a marked shoulder (Fig. 2). Transfection with the *ras* oncogene restored the shoulder to the dose-response curve of EGV 1 (Fig. 3), but did not alter the slope or shape of the dose-response relationship of EGV 9 (Fig. 4), to low-LET radiation.

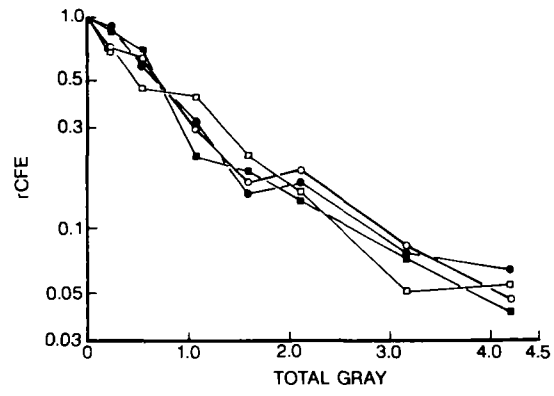

Figure 1. Cytotoxic response of EGV 1 or EGV 9 to α-particles. EGV 1 (□), EGV 1 + *ras* (○), EGV 9 (■), EGV 9 + *ras* (●).

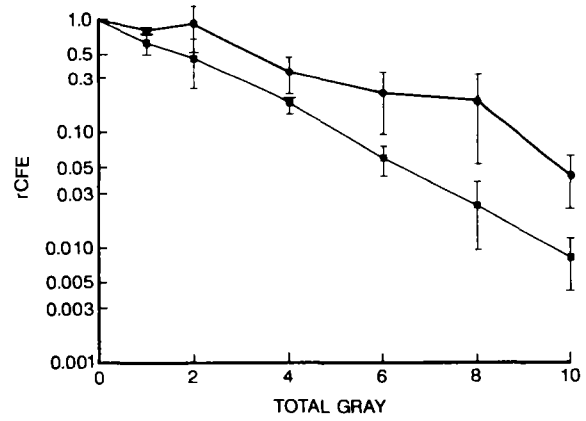

Figure 2. Cytotoxic response of EGV 1 (■) and EGV 9 (●) to X rays. Mean ± SE.

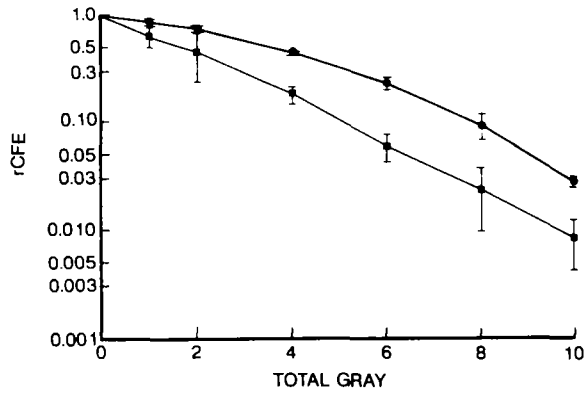

Figure 3. Cytotoxic response of EGV 1 (■) and EGV 1 + *ras* (●) to X rays. Mean ± SE.

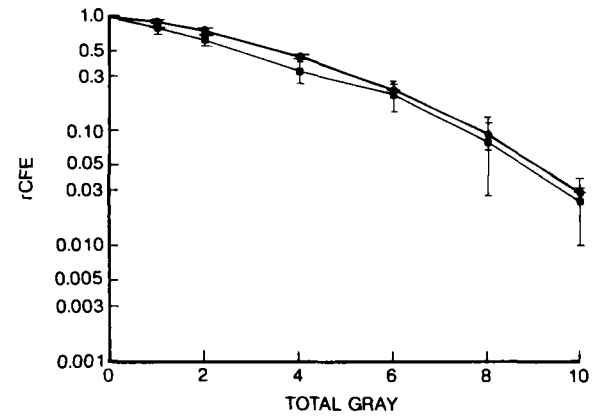

Figure 4. Cytotoxic response of EGV 9 (○) and EGV 9 + *ras* (■) to X rays. Mean ± SE.

DISCUSSION

Previous studies[1] have demonstrated that the sensitivity of a preneoplastic, fibroblastic cell line to X rays can be decreased following transfection of the cells with an activated *ras* oncogene. The results described here extend the previous observations to respiratory epithelial cells for both high- and low-LET radiation.

Three important observations were made. First, similar to the results with fibroblastic cells, the sensitivity of preneoplastic RTE cells to X rays can be altered by an activated *ras* oncogene. Second, the responsiveness of preneoplastic RTE cells to a *ras*-mediated reduction in X ray sensitivity depends on their responsiveness to *ras*-induced neoplastic transformation. Third, no differences in the responsiveness of the cells to high-LET radiation was observed with or without *ras* transformation.

The results described here suggest that the sensitivity of cells to X rays may change as cells progress through preneoplastic stages to neoplasia. Changes in the sensitivity of cells to X rays, and possibly to other agents, during the progression to neoplasia would result in a progressive change in the risk related to exposure to those agents. Such changes in sensitivity could serve as a basis for identifying specific stages of tumor development most affected by exposure to certain agents.

Alterations in the sensitivity of cells to X rays are most likely mediated through the ability of cells to repair radiation-induced damage. The observation that the *ras* oncogene can alter the shape of the dose-response curve suggests that this gene or its protein product are directly or indirectly involved in the repair of radiation-induced damage. The data presented here suggest that regulation of repair processes can change as cells progress to neoplasia.

Finally, previous studies have demonstrated that high-LET radiation-induced damage is poorly repaired compared with low-LET-induced damage. The studies described here support these observations. Cells were shown to differ in their sensitivities to low-LET, but not to high-LET, radiation. Transfection with a *ras* oncogene did not alter the cells' sensitivity to high-LET radiation.

These studies suggest that changes in the ability of cells to repair radiation damage may be related to their stage of progression to neoplasia. In addition, the capacity of cells to repair radiation-induced damage may be regulated by the *ras* oncogene or by genes with overlapping functional or regulatory domains.

REFERENCES

1. Sklar, M. D. The *Ras* Oncogenes Increase the Intrinsic Resistance of NIH3T3 Cells to Ionizing Radiation, *Science* **239**: 645-647, 1988.

2. Thomassen, D. G., T. E. Gray, M. J. Mass, and J. C. Barrett. High Frequency of Carcinogen-induced Early, Preneoplastic Changes in Rat Tracheal Epithelial Cells in Culture, *Cancer Res.* **43**: 5956-5963, 1983.

3. Thomassen, D. G., T. M. Gilmer, L. A. Annab, and J. C. Barrett. Evidence for Multiple Steps in Neoplastic Transformation of Normal and Preneoplastic Syrian Hamster Embryo Cells Following Transfection with Harvey Murine Sarcoma Virum Oncogene (v-*ras*), *Cancer Res.* **45**: 726-732, 1985.