

The Ras Radiation Resistance Pathway¹

Anjali K. Gupta, Vincent J. Bakanauskas,² George J. Cerniglia, Yi Cheng, Eric J. Bernhard, Ruth J. Muschel, W. Gillies McKenna

Departments of Radiation Oncology [A. K. G., V. J. B., G. J. C., E. J. B., W. G. M.] and Pathology and Laboratory Medicine [Y. C., R. J. M.], University of Pennsylvania, Philadelphia Pennsylvania 19104

ABSTRACT

The critical pathways determining the resistance of tumor cells to ionizing radiation are poorly defined. Because the *ras* oncogene, a gene activated in many human cancers treated with radiotherapy, can induce increased radioresistance, we have asked which Ras effector pathways are significant in conferring a survival advantage to tumor cells. The phosphoinositide-3-kinase (PI3K) inhibitor LY294002 radiosensitized cells bearing mutant *ras* oncogenes, but the survival of cells with wild-type *ras* was not affected. Inhibition of the PI3K downstream target p70S6K by rapamycin, the Raf-MEK-MAPK pathway with PD98059, or the Ras-MEK kinase-p38 pathway with SB203580 had no effect on radiation survival in cells with oncogenic *ras*. Expression of active PI3K in cells with wild-type *ras* resulted in increased radiation resistance that could be inhibited by LY294002. These experiments have indicated the importance of PI3K in mediating enhanced radioresistance and have implicated PI3K as a potential target for specific radiosensitization of tumors.

INTRODUCTION

The sensitivity of cells to killing by ionizing radiation is a critical, although not sole, determinant of the probability of cure in patients receiving radiotherapy for cancer as shown both in retrospective studies of cancer cell lines *in vitro* (1) and in prospective clinical trials (2). One factor known to increase tumor cell resistance to radiation is the presence of activated oncogenes. Hence, there has been considerable interest in determining which genes mediate altered radiosensitivity in tumor cells. The ability of the *ras* oncogene to lead to radioresistance has been indicated through several independent lines of experimentation. First, overexpression or transformation of rodent or human cells by Ras has been shown in many cases to result in cell lines that are substantially more resistant to radiation than the parental cells (3–5). In a second line of evidence, inhibition of Ras activation has resulted in radiosensitization both in rodent cells transfected with Ras (6) and in human tumor cell lines bearing endogenous mutations in Ras (3, 7). These studies have exploited independent methods to block Ras activation. Both lovastatin (3) and farnesyltransferase inhibitors (6, 7), which block the processing of Ras, result in radiosensitization. Similarly, transfection of cells expressing Ras with an adenoviral vector encoding an antisense Ras fragment leads to radiosensitization through the inhibition of Ras action (8). Finally, tumor cells with endogenous Ras activation are more resistant to radiation than are their counterparts in which the mutant *ras* oncogene has been eliminated by homologous recombination (9). Although there may be caveats about each of these lines of experimentation taken individually, together they offer a compelling case that expression of a mutant *ras* allele can alter the sensitivity of tumor cells and cause them to become more resistant to being killed by ionizing radiation. What

remains unclear from these experiments is which downstream mediators of Ras result in altered radiosensitivity.

Activation of Ras, either by mutation or through signaling from the cell-surface receptors, triggers a series of cascading activation of cytoplasmic kinases. The identification of the Ras signaling pathways that lead to radiation resistance thus becomes of considerable interest, because these pathways are potentially targets for manipulation of radiosensitivity in tumor cells with Ras mutations. Additionally, because signaling cascades may converge from multiple upstream mediators, identification of the downstream elements involved with Ras may give insights into cases in which radioresistance is seen in the absence of a Ras mutation. Of the Ras signaling pathways, the Ras-to-MAPK³ pathways are perhaps the most extensively studied; they consist of at least 3 sequential kinase cascades that include the Ras-Raf-MAPK (also known as extracellular regulated protein kinase) pathway, the stress-activated SAPK/JNK pathway, and the p38 pathway (10, 11). The Ras-to-MAPK pathway has in fact been implicated in radiosensitivity. Kasid *et al.* (12) transfected a truncated constitutively active *Raf* gene into a human squamous cell carcinoma cell line, leading to increased survival after radiation. The same group has also shown that down-regulation of Raf through antisense reduced that radioresistance of human cells (12, 13) implicating the Raf-MEK-MAPK pathway in radiation sensitivity. In addition, Kasid *et al.* (14) found that radiation itself induced Raf activation by colocalization of Ras and Raf to the inner cell membrane in cells with wild-type *ras*, although the physiological consequence of that induction has not yet been explored. Hagan *et al.* showed that MEK inhibition made DU145 cells, cells with wild-type *ras*, more radiosensitive and suggested that this was independent of apoptosis induction (15). In some cell lines with active Ras and wild-type p53, MEK inhibition might lead to radiosensitization through p53 induction (16). We have previously observed, however, that the inhibition of MAPK using the MEK inhibitor PD98059 failed to radiosensitize cells with activated Ras (17), which raises the possibility that another pathway would signal Ras-mediated radioresistance.

Ras also signals by directly binding and activating the catalytic p110 unit of PI3K (18). PI3K phosphorylates phosphatidylinositol (PtdIns) phosphates leading to the conversion of PtdIns 4,5-P₂ to PtdIns 3,4,5-P₃. This lipid second messenger activates the phosphoinositide-dependent kinases (PDK) PDK-1 and PDK-2, which then activate Akt, also known as protein kinase B (PKB). Additionally PI3K has other targets including p70S6K, Rac, and guanine exchange factors (19). PI3K has been implicated in mitogenic signaling, inhibition of apoptosis, intracellular vesicle trafficking, and secretion and regulation of actin and integrin functions (19–21). Ras also activates Rin and Ral by direct binding. Ral can activate CDC42 and Rac, which activate the various MEK kinase proteins that are involved in the regulation of the SAPK/JNK and p38 pathways (10, 11).

In this report, we explore the downstream pathway leading to

Received 1/3/01; accepted 3/13/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Supported by NIH Grants 1 PO-1 CA75138 (to W. G. M.), RO-1 GM47439 (to R. J. M.), American Society of Clinical Oncology Young Investigator Grant (to A. K. G.), and Radiological Society of North America Research Fellow Award (to A. K. G.).

² To whom requests for reprints should be addressed, at Department of Radiation Oncology, 195 John Morgan Building, 3620 Hamilton Walk, University of Pennsylvania, Philadelphia, PA 19104-6072.

³ The abbreviations used are: MAPK, mitogen-activated protein kinase; SAPK/JNK, MAP/ERK kinase; MEK, stress-activated protein kinase/Jun kinase; PI3K, phosphoinositide-3-kinase; EGF, epidermal growth factor; EGFR, EGF receptor; PTEN, phosphatase and tensin homologue.

radioresistance using two approaches. First, by pharmacological inhibition of Ras itself and potential downstream targets of Ras, we obtained results leading to the suggestion that PI3K was the potential downstream mediator. To confirm this, we then transiently expressed a constitutively active PI3K gene and showed that it could induce radioresistance like *ras*, and that this induction could similarly be pharmacologically inhibited.

MATERIALS AND METHODS

Cells. T24, RT4, and DLD-1 cells were obtained from American Type Culture Collection (Rockville, MD). The 3.7 and MR4 cells were derived from isogenic rat embryo fibroblasts by transfection with v-myc or v-myc + H-Ras^{V12} as described previously (4). Cells were cultured in DMEM (Fisher Scientific, Pittsburgh, PA) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Norcross, GA), penicillin (100 units/ml), and streptomycin (100 mg/ml; Life Technologies, Inc., Gaithersburg, MD) at 37°C in humidified 5% CO₂-95% air.

Cells were transfected with constitutively active PI3K consisting of the iSH2 domain of p85 fused to the NH₂ terminus of p110 by means of a flexible glycine linker (22) inserted into the pGRE5/EBV dexamethasone-inducible plasmid vector. Cells for transfection were harvested and suspended in electroporation buffer [2 mM HEPES, 15 mM K₂HPO₄/KH₂PO₄, 250 mM mannitol, 1 mM MgCl₂ (final pH 7.2)] at 3 × 10⁶ cells/ml. One hundred μl of the cell suspension, containing 4 μg of DNA, were electroporated with a Gene Pulser II system (Bio-Rad, Hercules, CA). The settings were optimized for both the MR4 and RT4 cells. For MR4, we used 140 V, 100% modulation, a DC amplitude of 70 V, a frequency of 10 KHz, a burst duration of 1.5 ms, and a total of 15 bursts with a burst interval of 1.5 s. The settings for RT4 cells were the same, except that the total number of bursts was decreased to seven. Cells were cultured, and 48 h later, 1 μg/ml of dexamethasone (Sigma Chemical Co., St. Louis, MO) was added. Radiation survival experiments were performed, and protein samples were harvested 24 h after the addition of dexamethasone.

Inhibitors. The MEK inhibitor PD98059, the PI3K inhibitor LY294002, the p70S6K inhibitor rapamycin, and the p38 inhibitors SB203580 and PD169316 were obtained from Alexis Corporation (San Diego, CA). The FTI L744,832 was obtained from Merck Pharmaceuticals (West Point, PA). All of the inhibitors were dissolved as concentrated stock solutions in DMSO and diluted at the time of treatment in medium. Control cells were treated with medium containing an equal concentration of DMSO.

Radiation Survival Determination. Cultures in log growth were counted and plated in 60-mm dishes containing 2 ml of medium. Inhibitors were added to cultures at least 1 h prior to radiation. L744,832 treatment was initiated 24 h prior to irradiation. Treatment was continued for 24 h after irradiation, at which time 3 ml of additional drug-free medium was added. Cells were irradiated with a Mark I cesium irradiator (J. L. Shepherd, San Fernando, CA) at a dose rate of 1.6 Gy/min. Colonies were stained and counted 10–14 days after irradiation. A light box was used to assist in counting all of the cell lines except RT4, which was counted with the aid of a microscope because, with radiation, the colonies tend to form giant cells; each colony was assessed individually to ascertain the presence of at least 50 cells. The surviving fraction was calculated as follows: number of colonies formed ÷ number of cells plated × plating efficiency. Each point on the survival curve represents the mean surviving fraction from at least three dishes.

Western Blotting. Cells were lysed without trypsinization by rinsing culture dishes once with PBS followed by lysis with reducing Laemmli sample buffer. Samples were boiled, sheared, and clarified by centrifugation and stored at -20°C. Samples containing equal amounts of protein were separated on a 12% SDS polyacrylamide gel and blotted onto nitrocellulose membranes. Membranes were blocked in PBS containing 0.1% Tween 20 and 5% powdered milk before the addition of primary antibody. Monoclonal H-Ras antibody LA069 (Quality Biotech) was used at a dilution of 1:5000; polyclonal antiphospho MAPK (Sigma Chemical Co.) was used at a dilution of 1:2000; polyclonal pan MAPK K-23 (Santa Cruz Biotechnology) was used at 1:500; and polyclonal antiphospho p38 MAPK, polyclonal antiphospho p70S6K, polyclonal antiphospho Ser 473 AKT, and polyclonal pan AKT (New England Biolabs) were all used at 1:2000 dilution. Antibody binding was detected using the ECL chemiluminescence kit (Amersham, Arlington Heights, IL). Images

were digitized using an Arcus II scanner, and figures were assembled using Adobe Photoshop 3.0 and Microsoft Power Point.

RESULTS

Pharmacological Survey in T24 Cells. To identify the Ras pathways that contribute to radiosensitization, we screened a panel of pharmacological agents known to block at specific points downstream of Ras (Fig. 1A). These agents were first examined using the cell line T24, a bladder cancer cell line that bears an oncogenic mutation in H-*ras*. T24 has been widely used as a model cell line for the study of *ras* oncogene-associated events, and we have shown that its degree of radiation resistance is Ras-dependent because inhibition of Ras processing by farnesyltransferase inhibitors leads to radiosensitization both *in vitro* (7) and *in vivo* (23). The dose of the pharmacological agents was chosen based on prior reports (24) and validated in T24 cells in dose-response experiments (data not shown). Inhibition of the phosphorylation of target proteins using the agents was confirmed (Fig. 1B). These same concentrations were then used for subsequent clonogenic survival assays. As expected in a cell line with endogenously activated H-Ras, T24 has high basal levels of phosphorylated p70S6K, Akt, p38, and MAPK. Inhibition of the Ras-Raf-MEK-MAPK pathway by the MEK inhibitor PD98059 (25 μM) blocked phosphorylation of MAPK but had no effect on p38, p70S6K, or Akt phosphorylation. SB203580 (25 μM) was used to specifically inhibit p38 phosphorylation. The drug PD169316, a putative p38 inhibitor,

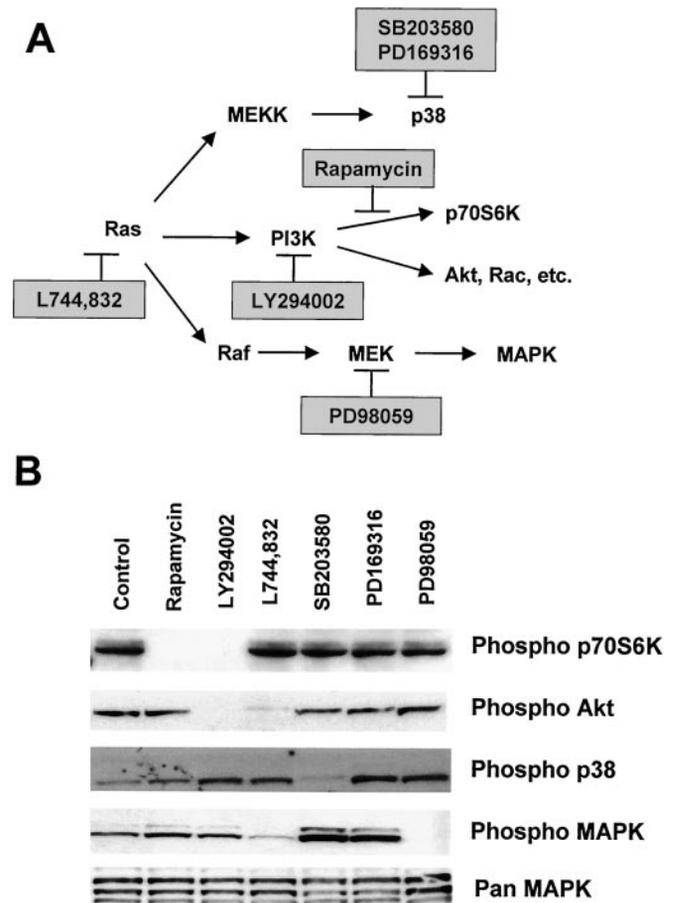


Fig. 1. Inhibitors known to block Ras signaling pathways. A, the Ras signal transduction pathway and the pharmacological inhibitors used. This is a simplistic schema, and significant cross-talk and additional and alternative pathways exist. B, inhibition of Ras signaling pathways in T24 cells. Signaling through Ras was evaluated by immunoblot analysis using antibodies with activity against the active or phosphorylated forms of p70S6K, Akt, p38, and MAPK. T24 cells were exposed to the indicated inhibitors.

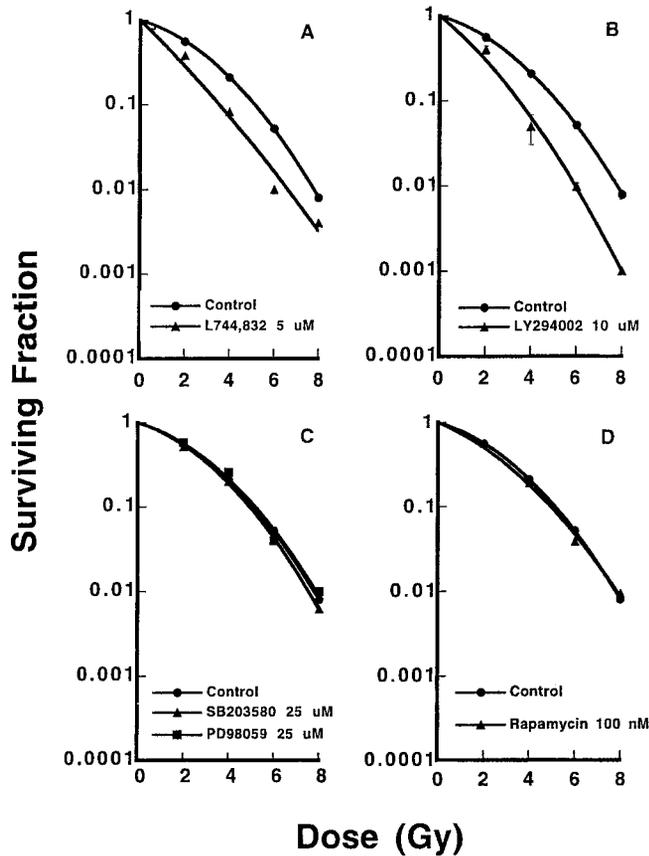


Fig. 2. Clonogenic survival in T24 cells after treatment with inhibitors of the Ras signal transduction pathway. Survival after treatment with A, FTI L744,832 (\blacktriangle); B, LY294002 (\blacktriangle); C, SB203580 (\blacktriangle), PD98059 (\blacksquare); and D, rapamycin (\blacktriangle). The control curve (\bullet) in all of the panels is the same. The error bars are shown and, if not visible, were contained within the point of the graph.

failed to inhibit p38 phosphorylation in our hands, and its further use was not pursued. LY294002 is a PI3K inhibitor and, as predicted from the scheme outlined in Fig. 1A, inhibited the phosphorylation of both Akt and p70S6K, but not of MAPK or p38. Rapamycin specifically inhibited p70S6K phosphorylation. Inhibition of Ras farnesylation by 24-h pretreatment with 5 μ M FTI L744,832 is known to radiosensitize T24 cells (7, 23) and resulted in decreased MAPK and Akt phosphorylation, although complete inhibition was not obtained. This treatment did not affect p70S6K or p38 phosphorylation, which suggests that the activation of these proteins can proceed via alternative pathways in T24 cells (Fig. 1B).

Having verified the efficacy and specificity of the Ras pathway inhibitors, we examined their effect on the clonogenic survival of T24 cells. The FTI L744,832 was confirmed to reduce clonogenic survival after irradiation (Fig. 2A), as reported previously (7, 23). Inhibition of PI3K by LY294002 sensitized the T24 cells to ionizing radiation by the same extent (Fig. 2B). In contrast, neither inhibition of p38 nor inhibition of MEK-MAPK affected the radiosensitivity of these cells (Fig. 2C). The p70S6K inhibitor, rapamycin, also failed to radiosensitize (Fig. 2D). These results suggested that PI3K has an important contribution in Ras-mediated radiation resistance in T24 cells.

PI3 Kinase Inhibition in Other Cell Lines. To generalize the findings obtained in T24 cells, we examined the effect of the inhibitor panel on the clonogenic survival of cells with varying *ras* mutation status (Fig. 3A). Akt phosphorylation, which indicated PI3K activity, was evident in both of the cell lines with *ras* mutations, 3.7 (H-Ras^{V12}) and DLD-1 (K-Ras^{D13}). This phosphorylation was apparent under basal conditions and was augmented after irradiation without

changes in Akt protein levels. The extent of Akt phosphorylation was sensitive to cell culture conditions. Confluence resulted in phosphorylation of Akt that was not further induced by radiation (data not shown). Treatment with LY294002 completely inhibited both basal (data not shown) and radiation-induced Akt phosphorylation in both of these cell lines (Fig. 3B). Clonogenic survival was reduced by PI3K inhibition in both DLD-1 and 3.7 cells (Fig. 4, A and B). In contrast, RT4 and MR4 cell lines, expressing wild-type *ras*, showed little detectable basal or radiation-induced Akt phosphorylation (Fig. 3C). Inhibition of PI3K also had no effect on the radiation survival of either of these two cell lines (Fig. 4, C and D). Clonogenic survival was also not affected by MEK-MAPK inhibition using PD98059 in the cell lines examined (previously published and data not shown; Refs. 16, 17, 25). Taken together, the pharmacological data pointed to PI3K as a significant mediator of Ras-induced radiation resistance.

Effect of PI3K Induction. To examine the hypothesis that PI3K activity is an important contributor to radiation survival, both of the cell lines expressing wild-type *ras* (MR4 and RT4) were transfected with a constitutively active PI3K under control of a dexamethasone-inducible promoter. The catalytic subunit of PI3K, p110, exhibits enzymatic activity when bound through its NH₂-terminal region to the p85 subunit (19), which allows the construction of a constitutively active PI3K consisting of the iSH2 region of p85 covalently attached to the NH₂ terminus of the p110 subunit (22). Dexamethasone treatment of these transiently transfected cells resulted in the appearance of phosphorylated Akt in both MR4 (Fig. 5A) and RT4 (Fig. 5B) cells that was inhibited by treatment with LY294002. The presence of phosphorylated Akt as a marker for PI3K activity was specific for

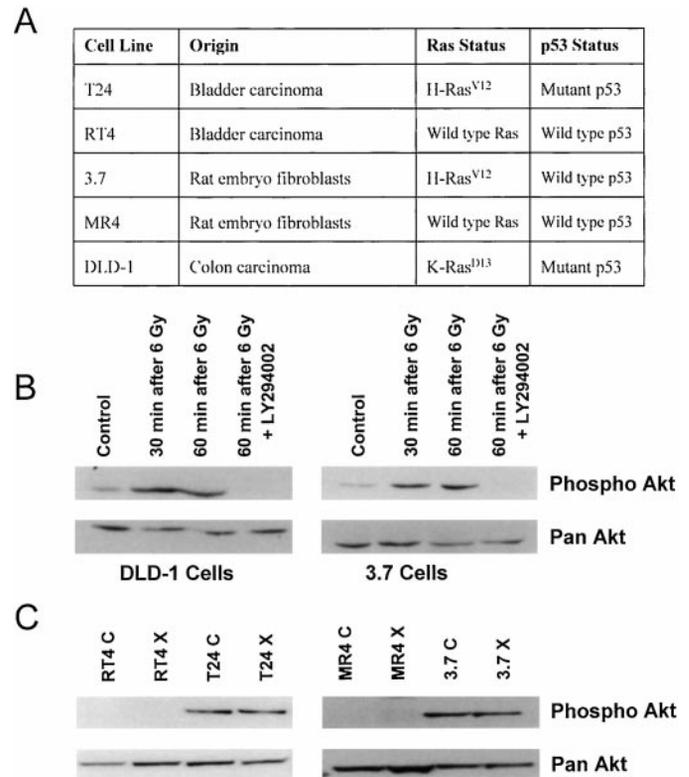


Fig. 3. PI3K activation in cell lines with differing Ras mutations. A, table indicating all of the cells tested. We surveyed four other cell lines in addition to T24 with known mutations in *ras* or p53. B, activation of PI3K in two cell lines with activating mutations in Ras (DLD-1 and 3.7). The endogenous activity of PI3K and the activity of PI3K after treatment with the PI3K inhibitor LY294002 was evaluated by monitoring the presence of phospho Akt using a specific antibody. The same gels were stripped and probed with pan-Akt. C, Akt activation in the two cell lines with wild-type *ras* (RT4 and MR4) before (RT4C and MR4C) and after irradiation (RT4X and MR4X). The gel was first probed with the antiphospho Akt antibody and was then stripped and probed with pan-Akt.

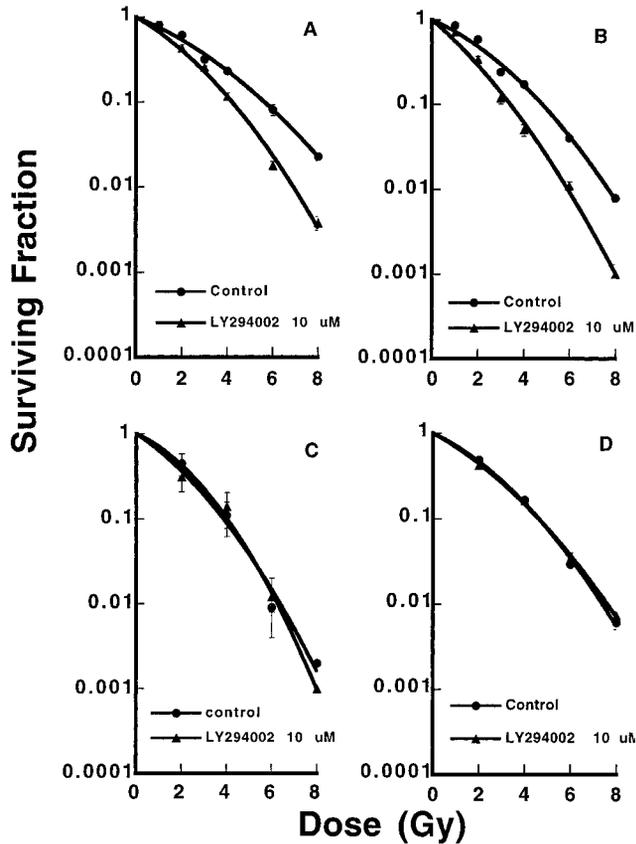


Fig. 4. Clonogenic survival in cell lines with differing Ras mutation after treatment with the PI3K inhibitor LY294002. The control (●) and the LY294002-treated (▲) curve in A, DLD-1 cells; B, 3.7 cells; C, MR4 cells; D, and RT4 cells. The error bars are shown and, if not visible, were contained within the point on the graph.

cells expressing the activated p110 subunit because neither untransfected cells treated with dexamethasone nor uninduced transfectants showed enhanced Akt phosphorylation. The effect of expressing active PI3K on radiation survival was then examined in these cells. In both MR4 and RT4, cells transfected with active PI3K that was treated

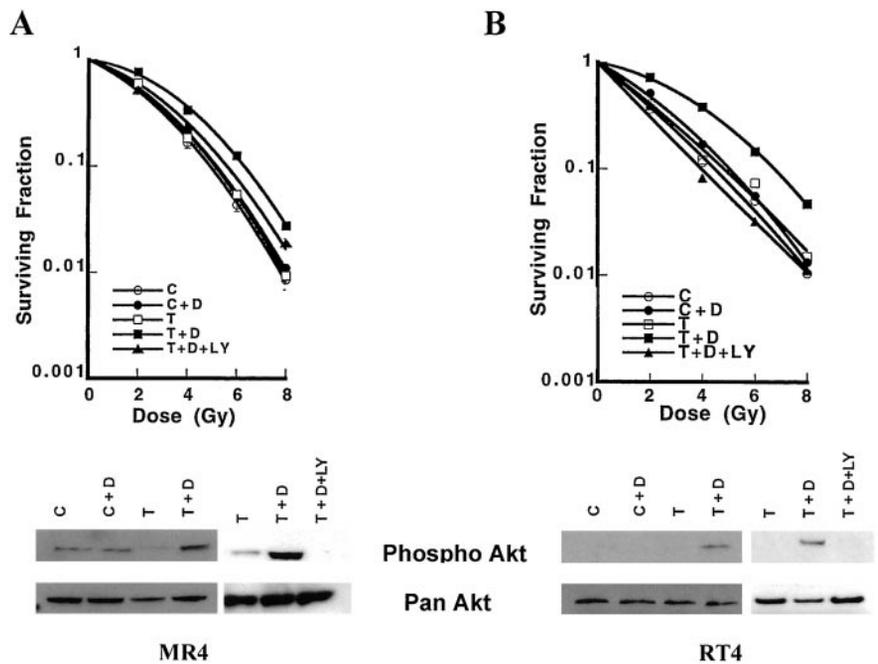
with dexamethasone had enhanced clonogenic survival after irradiation (Fig. 5). No alteration in survival was seen in control cells treated with dexamethasone nor in transfectant cells in the absence of dexamethasone. Expression of the vector and exposure to dexamethasone did not alter the plating efficiency of the cells. In addition, treatment of transfected cells with LY294002 in the presence of dexamethasone sensitized them to radiation.

DISCUSSION

Using two different approaches, we have identified PI3K as an important mediator of Ras-induced radiation resistance. Using the PI3K inhibitor LY294002, we observed radiosensitization of cell lines with active PI3K and activating *ras* mutations, but not cells with wild-type *ras* and inactive PI3K, which is consistent with PI3K signaling mediating Ras-induced radioresistance. In a second approach, we expressed constitutively active PI3K in cell lines with wild-type *ras* and showed induction of radiation resistance. This induction was also inhibited by LY294002. LY294002 is not completely specific for PI3K, and the possibility of inhibition of activity of PI3K-like proteins, such as ATM or DNA-PK, must be considered. The K_i value for DNA-PK of LY294002 is $6 \mu\text{M}$ (26, 27); therefore, at $10 \mu\text{M}$ LY294002, we would inhibit 70% of DNA-PK activity. Whether this level of inhibition would alter survival is not known. The K_i of LY294002 against ATM is not known. However, arguing against these PI3K-like proteins as targets was the failure of LY294002 to sensitize cell lines with wild-type *ras*, in which case, both DNA-PK and ATM would have been equally affected. Furthermore the enhanced radiation survival after transfection with a constitutively active PI3K and subsequent sensitization by LY294002 is not consistent with either DNA-PK or ATM mediating radiation resistance nor being the targets of LY294002 for this effect.

Other Ras pathways have been implicated in radiation resistance. Activation of Raf led to radiation resistance, and its inhibition by antisense constructs sensitized cells from head and neck carcinomas (12, 28). The inhibition of MAPK can also sensitize some cell lines (15). However, in the cell lines examined here, inhibition of the Ras-Raf-MEK-MAPK pathway with PD98059 did not result in sensitization. The Ras-MAPK pathway may effect survival in a p53-

Fig. 5. Resistance to radiation is conferred by constitutively active PI3K. Cells were transfected by electroporation with an expression vector coding for a constitutively active PI3K p110 under control of a dexamethasone responsive promoter. Clonogenic survival in A, MR4 cells and B, RT4 cells with no treatment (○), dexamethasone alone (●), transfection alone (□), transfection plus dexamethasone (■), and transfection plus dexamethasone and LY294002 (▲). Western blot analysis was also performed on protein samples harvested at the time of the survival curve. The lysates from cells with no treatment (C), dexamethasone alone (C + D), transfection alone (T), transfection plus dexamethasone (T + D), and transfection plus dexamethasone and LY294002 (T + D+LY) were probed with the antiphospho Akt and pan-Akt antibodies.



dependent manner through induction of p19ARF, a transcription factor that increases expression of Mdm2, a protein that binds to p53 and signals its destruction (16). In some cells with wild-type p53, the inhibition of MAPK can result in radiosensitization. However, this pathway can be countered through mutations in p19ARF or overexpression of Mdm2, both of which can reduce p53 levels (29). In the cell lines studied here, 3.7, MR4, and RT4 are wild type for p53 (Fig. 3A), but their clonogenic survival after inhibition of MAPK was not altered. Thus, although the Raf-MAPK pathway may also be a mediator of altered radiosensitivity in some systems, our conclusion is that it is not the downstream mediator of Ras-induced radioresistance.

The identification of PI3K as the downstream mediator of Ras-induced radioresistance is of considerable interest, because the PI3K pathway may also affect radioresistance independently of Ras activation, consistent with our hypothesis that there may be final common pathways that converge in the induction of radioresistance. EGF can signal through PI3K (30). Hence, the overexpression of EGFR, which is frequently seen in cancers, might lead to increased PI3K activity. An association between EGFR and clinical radioresistance has been reported in patients with head and neck cancers (31) and astrocytic gliomas (32). Mutations causing PTEN to be functionally inactive are frequently found in many human cancers. PTEN is a phosphatase that antagonizes PI3K by converting its active product PI(3,4,5)P3 to PI(4,5)P2 (33). Tumor cells with these mutations may have augmented PI3K activity and, hence, be susceptible to radiosensitization by PI3K inhibition. Wick *et al.* (34) have shown that PTEN gene transfer in human malignant gliomas sensitized cells to radiation, although in this case PTEN transfer was associated with growth suppression, which may complicate the interpretation. Many tumors with mutations in Ras or PTEN or tumors that have up-regulated EGFR are treated with radiation therapy. Identification of a common downstream signal that leads to radiation resistance may uncover targets for developing molecular-based radiosensitization protocols for tumors resistant to radiation and, thus, improve the local control that can be obtained after radiation therapy.

REFERENCES

- Girinsky, T., Lubin, R., Pignon, J. P., Chavaudra, N., Gazeau, J., Dubray, B., Cosset, J. M., Socie, G., and Fertel, B. Predictive value of *in vitro* radiosensitivity parameters in head and neck cancers and cervical carcinomas: preliminary correlations with local control and overall survival. *Int. J. Radiat. Oncol. Biol. Phys.*, 25: 3-7, 1992.
- West, C. M. L., Davidson, S. E., Roberts, S. A., and Hunter, R. D. Intrinsic radiosensitivity and prediction of patient response to radiotherapy for carcinoma of the cervix. *Br. J. Cancer*, 68: 819-823, 1993.
- Miller, A. C., Kariko, K., Myers, C. E., Clark, E. P., and Samid, D. Increased radioresistance of EJras-transformed human osteosarcoma cells and its modulation by lovastatin, an inhibitor of p21^{ras} isoprenylation. *Int. J. Cancer*, 53: 302-307, 1993.
- McKenna, W. G., Weiss, M. A., Bakanauskas, V. J., Sandler, H., Kelsten, M., Biaglow, J., Endlich, B., Ling, C., and Muschel, R. J. The role of the HRAS oncogene in radiation resistance and metastasis. *Int. J. Radiat. Oncol. Biol. Phys.*, 18: 849-860, 1990.
- Ling, C. C., and Endlich, B. Radioresistance induced by oncogenic transformation. *Radiat. Res.*, 120: 267-279, 1989.
- Bernhard, E. J., Kao, G., Cox, A. D., Sebt, S. M., Hamilton, A. D., Muschel, R. J., and McKenna, W. G. The farnesyltransferase inhibitor FTI-277 radiosensitizes H-Ras-transformed rat embryo fibroblasts. *Cancer Res.*, 56: 1727-1730, 1996.
- Bernhard, E. J., McKenna, W. G., Hamilton, A. D., Sebt, S. M., Qian, Y., Wu, J. M., and Muschel, R. J. Inhibiting Ras prenylation increases radiosensitivity of human tumor cell lines with activating mutations of Ras oncogenes. *Cancer Res.*, 58: 1754-1761, 1998.
- Rait, A., Pirolo, K., Will, D. W., Peyman, A., Rait, V., Uhlmann, E., and Chang, E. H. 3'-end conjugates of minimally phosphorothioate-protected oligonucleotides with 1-O-hexadecylglycerol: synthesis and anti-ras activity in radiation-resistant cells. *Bioconjug. Chem.*, 11: 153-160, 2000.
- Bernhard, E. J., Stanbridge, E. J., Gupta, S., Gupta, A. K., Soto, D., Bakanauskas, V. J., Cerniglia, G. J., Muschel, R. J., and McKenna, W. G. Direct evidence for the contribution of activated N-ras and K-ras oncogenes to increased intrinsic radiation resistance in human tumor cell lines. *Cancer Res.*, 60: 6597-6600, 2000.
- Reuther, G. W., and Der, C. J. The Ras branch of small GTPases: ras family members don't fall far from the tree. *Curr. Opin. Cell Biol.*, 12: 157-165, 2000.
- Elion, E. A. Routing MAP kinase cascades. *Science (Wash. DC)*, 281: 1625-1626, 1998.
- Kasid, U., Pfeifer, A., Brennan, T., Beckett, M., Weichselbaum, R. R., Dritschilo, A., and Mark, G. E. Effect of antisense c-raf-1 on tumorigenicity and radiation sensitivity of a human squamous carcinoma. *Science (Wash. DC)*, 243: 1354-1356, 1989.
- Gokhale, P. C., McRae, D., Monia, B. P., Bagg, A., Rahman, A., Dritschilo, A., and Kasid, U. Antisense raf oligodeoxynucleotide is a radiosensitizer *in vivo*. *Antisense Nucleic Acid Drug Dev.*, 9: 191-201, 1999.
- Kasid, U., Suy, S., Dent, P., Ray, S., Whiteside, T. L., and Sturgill, T. W. Activation of raf by ionizing radiation. *Nature (Lond.)*, 382: 813-816, 1996.
- Hagan, M., Wang, L., Hanley, J. R., Park, J. S., and Dent, P. Ionizing radiation induced mitogen activated protein kinase activation in DU145 prostate carcinoma cells: MAP kinase inhibition enhances radiation-induced cell killing and G₂/M phase arrest. *Radiat. Res.*, 153: 371-383, 2000.
- Ries, S., Biederer, C., Woods, D., Shifman, O., Shrasawa, S., Sasazuki, T., McMahon, M., Oren, M., and McCormick, F. Opposing effects of Ras on p53: transcriptional activation of mdm2 and induction of p19ARF. *Cell*, 103: 321-330, 2000.
- Gupta, A. K., Bernhard, E. J., Bakanauskas, V. J., Wu, J. M., Muschel, R. J., and McKenna, W. G. RAS-mediated radiation resistance is not linked to MAP kinase activation in two bladder carcinoma cell lines. *Radiat. Res.*, 154: 64-72, 2000.
- Rodriguez-Viciano, P., Warne, P. H., Dhand, R., Vanhaesebroeck, B., Gout, I., Fry, M. J., Waterfield, M. D., and Downward, J. Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature (Lond.)*, 370: 527-532, 1994.
- Carpenter, C. L., and Cantley, L. C. Phosphoinositide kinases. *Curr. Opin. Cell Biol.*, 8: 153-158, 1996.
- Rasilnikov, M. K., Adler, V., Fuchs, S. Y., Dong, Z., Haimovitz-Friedman, A., Herlyn, M., and Ronai, Z. Contribution of phosphatidylinositol 3-kinase to radiation resistance in human melanoma cells. *Mol. Carcinog.*, 24: 64-69, 1999.
- Gire, V., Marshall, C., and Wynford-Thomas, D. PI-3-kinase is an essential antiapoptotic effector in the proliferative response of primary human epithelial cells to mutant RAS. *Oncogene*, 19: 2269-2276, 2000.
- Hu, Q., Klippel, A., Muslin, A. J., Fantl, W. J., and Williams, L. T. Ras-dependent induction of cellular responses by constitutively active phosphatidylinositol-3 kinase. *Science (Wash. DC)*, 268: 100-102, 1995.
- Cohen-Jonathan, E., Muschel, R. J., McKenna, W. G., Evans, S. M., Cerniglia, G., Mick, R., Kusewitt, D., Sebt, S. M., Hamilton, A. D., Oliff, A., Kohl, N., Gibbs, J. B., and Bernhard, E. J. Farnesyltransferase inhibitors potentiate the antitumor effect of radiation on a human tumor xenograft expressing activated HRAS. *Radiat. Res.*, 154: 125-132, 2000.
- Davies, S. P., Reddy, H., Caivano, M., and Cohen, P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.*, 351: 95-105, 2000.
- Belka, C., Knippers, P., Rudner, J., Faltin, H., Bamberg, M., and Budach, W. MEK1 and Erk1/2 kinases as targets for the modulation of radiation responses. *Anticancer Res.*, 20: 3243-3249, 2000.
- Rosenzweig, K. E., Youmell, M. B., Palayoor, S. T., and Price, B. D. Radiosensitization of human tumor cells by the phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 correlates with inhibition of DNA dependent protein kinase and prolonged G₂-M delay. *Clin. Cancer Res.*, 3: 1149-1156, 1997.
- Izzard, R. A., Jackson, S. P., and Smith, G. C. M. Competitive and noncompetitive inhibition of the DNA-dependent protein kinase. *Cancer Res.*, 59: 2581-2586, 1999.
- Pfeifer, A., Mark, G., Leung, S., Dougherty, M., Spillare, E., and Kasid, U. Effects of c-raf-1 and c-myc expression on radiation response in an *in vitro* model of human small cell lung carcinoma. *Biochem. Biophys. Res. Commun.*, 253: 481-486, 1998.
- Sherr, C. J. The Pezcoller lecture: cancer cell cycle revisited. *Cancer Res.*, 60: 3689-3695, 2000.
- Okano, J., Gaslightwala, I., Birnbaum, M. J., Rustgi, A. K., and Nakagawa, H. Akt/protein kinase B isoforms are differentially regulated by epidermal growth factor stimulation. *J. Biol. Chem.*, 275: 30934-30942, 2000.
- Maurizi, M., Almadori, G., Ferrandina, G., Distefano, M., Romanini, M. E., Cadoni, G., Benedetti-Panici, P., Paludetti, G., Scambia, G., and Mancuso, S. Prognostic significance of epidermal growth factor receptor in laryngeal squamous cell carcinoma. *Br. J. Cancer*, 74: 1253-1257, 1996.
- Zhu, A., Shaeffer, J., Leslie, S., Kolm, P., El-Mahdi, A. M. Epidermal growth factor receptor: an independent predictor of survival in astrocytic tumors given definitive irradiation. *Int. J. Radiat. Oncol. Biol. Phys.*, 34: 809-815, 1996.
- Urnisa, I., Schriml, L. M., and Dean, M. Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. *J. Natl. Cancer Inst. (Bethesda)*, 91: 1922-1932, 2000.
- Wick, W., Furnari, F. B., Naumann, U., Cavenee, W. K., and Weller, M. PTEN gene transfer in human malignant glioma: sensitization to irradiation and CD95L-induced apoptosis. *Oncogene*, 18: 3936-3943, 1999.