Role of Cell Cycle in Epidermal Growth Factor Receptor Inhibitor-Mediated Radiosensitization

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Abstract

Epidermal growth factor receptor (EGFR) inhibitors are increasingly used in combination with radiotherapy in the treatment of various EGFR-overexpressing cancers. However, little is known about the effects of cell cycle status on EGFR inhibitor-mediated radiosensitization. Using EGFR-overexpressing A431 and UM1SCC-1 cells in culture, we found that radiation activated the EGFR and extracellular signal-regulated kinase pathways in quiescent cells, leading to progression of cells from G1 to S, but this activation and progression did not occur in proliferating cells. Inhibition of this activation blocked S-phase progression and protected quiescent cells from radiation-induced death. To determine if these effects were caused by EGFR expression, we transected Chinese hamster ovary (CHO) cells, which lack EGFR expression, with EGFR expression vector. EGFR expressed in CHO cells also became activated in quiescent cells but not in proliferating cells after irradiation. Moreover, quiescent cells expressing EGFR underwent increased radiation-induced clonogenic death compared with both proliferating CHO cells expressing EGFR and quiescent wild-type CHO cells. Our data show that radiation-induced enhancement of cell death in quiescent cells involves activation of the EGFR and extracellular signal-regulated kinase pathways. Furthermore, they suggest that EGFR inhibitors may protect quiescent tumor cells, whereas radiosensitization of proliferating cells may be caused by downstream effects such as cell cycle redistribution. These findings emphasize the need for careful scheduling of treatment with the combination of EGFR inhibitors and radiation and suggest that EGFR inhibitors might be best given after radiation in order to optimize clinical outcome.

Introduction

Inhibitors of the epidermal growth factor receptor (EGFR) have shown considerable promise when used alone and in combination with chemotherapy and radiation therapy (1). In particular, the outcome of treatment with the combination of EGFR inhibition and radiation therapy in preclinical studies (2–6) and in a randomized clinical trial in head and neck cancer (7, 8) is superior to that of radiation therapy alone. These results have fueled interest in optimizing the interaction of EGFR inhibition with radiation therapy.

As tumors are composed of proliferating and quiescent cells, understanding the effects of the proliferation status of cells could represent a key aspect in optimizing EGFR-radiation interactions. Schmidt-Ullrich and colleagues found that radiation can stimulate EGFR phosphorylation in serum-starved, growth-arrested cells (with low background EGFR levels). If this EGFR stimulation were part of a survival signal, EGFR inhibitors could sensitize cells to radiation (9). Conversely, EGFR stimulation via its ligand could stimulate G0 quiescent cells to enter S phase. If this occurred in the presence of unrepaired radiation-induced DNA damage, it could increase radiation sensitivity. In this case, EGFR inhibition might actually be radioprotective.

EGFR inhibitors might interact quite differently with irradiated proliferating cells. These cells have high levels of EGFR activation at baseline, and the additional stimulation by radiation appears to have little effect on this already high baseline level (10). However, prolonged exposure to EGFR inhibitors can place cells in a relatively sensitive phase of the cell cycle, late G1, which can increase radiation sensitivity (11).

To better understand the effects of EGFR inhibitors on radiosensitivity with respect to cellular proliferation status, we compared the effects of radiation on EGFR-overexpressing cells that were proliferating versus quiescent cells arrested in G0-G1. In the case of quiescent cells, we hypothesized that radiation would cause stimulation of EGFR, over low baseline levels, which would activate extracellular signal-regulated kinase (ERK) and drive cells into S-phase. This would lead to a decrease in DNA repair and increased radiation-induced cell death. In contrast, we anticipated that proliferating cells would not exhibit increased phosphorylation of EGFR over already high baseline levels (10); therefore, proliferating cells might be relatively resistant to radiation compared with quiescent cells in G0-G1. When we found that this was the case, we developed a system using wild-type Chinese hamster ovary (CHO) cells that do not express EGFR (12) and CHO cells that were transfected with the EGFR expression vector. This system permitted us to specifically determine the role of radiation-induced EGFR activation and downstream stimulation on radiation sensitivity and to understand the difference in radiation sensitivity between proliferating and quiescent cells whose growth is driven by EGFR.

Materials and Methods

Reagents. Phospho-EGFR (pEGFR; Y845), phospho-ERK (pERK; T202/ Y204), GAPDH and total ERK antibodies, and U0126 were purchased from Cell Signaling Technology. EGFR antibody (sc-03) was acquired from Santa Cruz Biotechnology. γ-H2AX antibody was acquired from Upstate. EGF was purchased from Sigma. Erlotinib was kindly provided by Genentech.

Cell culture. CHO-K1 and A431 cell lines were purchased from the American Type Culture Collection. The human head and neck squamous cell carcinoma cell line UM1SCC-1 was a gift from Dr. Thomas E. Carey (University of Michigan). All cell lines were grown in RPMI 1640 supplemented with 10% cosmic calf serum (Hyclone). Experiments were conducted in
serum-containing medium. For in vitro experiments, cells were released from flasks using PBS containing 0.01% trypsin and 0.20 mmol/L EDTA and plated 2 days before treatment. For experiments with proliferating cells, \(6 \times 10^5\) cells were plated in 10 cm Petri dishes in 10 mL medium, and the cultures were between 30% and 50% confluent at the time of harvest. For quiescent culture, \(10^6\) cells were plated in 10 cm Petri dishes in 10 mL medium; once the culture plate reached \(\sim 90\%\) confluence (\(\sim 5-7\) days after seeding), the growth medium was replaced. Two days later, cells were irradiated and analyzed.

**Radiation and drug treatment.** Cells were irradiated at room temperature at a dose rate of 3 Gy/min using a Pantak DXT300 orthovoltage unit. Dosimetry was carried out using an ionization chamber connected to an electrometer system that was directly traceable to a National Institute of Standards and Technology calibration. For drug treatment, cells were briefly exposed to 10 μmol/L U0126 (1 h), 10 ng/mL EGF (30 min), or 3 μmol/L erlotinib (2 h). Growth medium was replaced following drug treatment.

**Flow cytometry.** Cells were harvested and fixed in 70% ethanol. For DNA content flow cytometry, cells were stained with a solution of 0.018 mg/mL propidium iodide and 0.04 mg/mL RNase A. For bromodeoxyuridine (BrdUrd) flow cytometry, cells were exposed to 30 μmol/L BrdUrd for 15 min and processed as described previously (13) using an antibody recognizing BrdUrd (Pharmingen) followed by a FITC-conjugated goat anti-mouse secondary antibody (Sigma). In each experiment, a control sample without BrdUrd was processed to determine the background signal. Ten thousand or 40,000 cells were analyzed using on a Beckman Coulter Epics Elite or a Becton Dickinson FACScan, respectively. The graphs were generated using WinMDI software.

**Immunoblotting.** Cells were scraped into PBS containing sodium orthovanadate and a protease inhibitor mixture (Complete Protease Inhibitor; Roche Diagnostic). Cells were incubated for 15 min on ice in Laemml buffer [63 mmol/L Tris-HCl, 2% (w/v) SDS, 10% (v/v) glycerol, and 0.005% (w/v) bromophenol blue] containing 100 mmol/L NaF, 1 mmol/L Na3VO4, 1 mmol/L phenylmethylsulfonyl fluoride, and 1 μg/mL aprotinin. After sonication, particulate material was removed by centrifugation at 13,000 rpm for 15 min at 4°C. The soluble protein fraction was heated to 95°C for 5 min and then applied to a 4% to 12% bis-Tris precast gel (Invitrogen) and transferred onto a polyvinylidene difluoride membrane. Membranes were incubated for 1 h at room temperature in blocking buffer consisting of 3% bovine serum albumin and 1% normal goat serum in TBS [137 mmol/L NaCl, 20 mmol/L Tris-HCl (pH 7.6), 0.1% (v/v) Tween 20]. Membranes were subsequently incubated overnight at 4°C with 1 μg/mL primary antibody in blocking buffer, washed, and incubated for 1 h with horseradish peroxidase-conjugated secondary antibody (Cell Signaling). After three additional washes in TBS, bound antibody was detected by enhanced chemiluminescence plus reagent (Amersham Biosciences). For quantification of relative

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**Figure 1.** Effect of cell culture condition on the radiation sensitivity of A431 and UMSCC-1 cells. A, proliferating and quiescent A431 and UMSCC-1 cells were exposed to various doses of radiation and plated for clonogenic survival assays. Quiescent cells were more sensitive to radiation than proliferating cells (ratio of surviving fractions = 2.70 at 4 Gy). B, cells were treated with 4 Gy and harvested at various time points after radiation. Levels of pEGFR, EGFR, pERK1/2, ERK1/2, γ-H2AX, and GAPDH proteins were measured in the cell lysates. Immediate activation of EGFR and ERK (by 5 min) was induced in quiescent cells but not in proliferating cells. DNA damage in proliferating cells was repaired by 2 h but remained unrepaired in quiescent cells. RT, radiation; Conf, confluent; Log, logarithmic.

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Results

Radiosensitivity and growth factor signaling in quiescent and proliferating cells. We selected UMSCC-1 human head and neck cancer cells and A431 human epithelial cancer cells for study, as both are driven by EGFR overexpression (15). We first sought to determine the relative radiation sensitivity of quiescent and proliferating A431 cells. We found that quiescent A431 cells (G1 ≥ 80%) were more sensitive to radiation than proliferating A431 cells (G1 ≤ 50%; enhancement ratio = 1.89 ± 0.02; P < 0.001; Fig. 1A). Similar experiments in UMSCC-1 cells confirmed that quiescent cells were more radiosensitive than proliferating cells (enhancement ratio = 1.70 ± 0.01; P < 0.001; Fig. 1A). Next, we used the EGFR inhibitor erlotinib to assess the contribution of EGFR activation to radiation sensitivity in quiescent and proliferating cells, reasoning that a brief exposure to drug would inhibit pEGFR without confounding effects such as cell cycle changes (10, 16). We found that a 2-h pretreatment with 3 μmol/L erlotinib significantly protected quiescent cells (enhancement ratio = 0.67 ± 0.01) and also slightly but significantly protected proliferating cells (enhancement ratio = 0.91 ± 0.04). Erlotinib also significantly protected UMSCC-1 quiescent cells (enhancement ratio = 0.78 ± 0.05) and, to a lesser degree, proliferating cells (enhancement ratio = 0.89 ± 0.07). These data support our hypothesis that EGFR-driven, quiescent cells are more radiosensitive than proliferating cells and that EGFR inhibition is radioprotective, particularly in quiescent cells.

We next sought to determine if EGFR and ERK signaling were differentially affected after irradiation in quiescent and proliferating A431 and UMSCC-1 cells. In quiescent cells, irradiation induced
immediate (5 min) EGFR and ERK activation, both of which returned to normal levels by 2 h (Fig. 1B). In contrast, there was no radiation-induced EGFR and ERK phosphorylation in proliferating cells; in fact, pEGFR and pERK levels were decreased as we have reported previously (Fig. 1B; ref. 10). We also noticed that the unresolved DNA lesions in quiescent UMSSC-1 cells as reflected by high γ-H2AX levels were prolonged, as γ-H2AX levels had not decreased by 2 h after the initial spike in UMSSC-1 cells (Fig. 1B). In contrast, γ-H2AX levels in irradiated proliferating cells were resolved within 2 h. These findings show that, in EGFR-driven cells, irradiation induces EGFR-ERK signaling and unrepaired DNA damage in quiescent cells, but these effects were not found in proliferating cells.

We then investigated the effect of radiation on cell cycle distribution of quiescent and proliferating A431 cells after irradiation (4 Gy). One parameter flow cytometry showed an increase in apoptotic cells as reflected by an increase in sub-G1 content in G0 cells compared with proliferating cells 24 h after irradiation (Fig. 2A). This analysis also revealed that radiation caused a significant movement of cells with G1 DNA content into S in the initially quiescent cell population (percent in S phase before and after radiation was 8 ± 3% and 28 ± 6%, respectively), whereas initially proliferating cells did not undergo this movement after irradiation (percent in S phase before and after radiation was 31 ± 5% and 15 ± 3%, respectively; Fig. 2A). To further assess the significance of the response to radiation with regard to cell cycle progression in quiescent cells, we examined the effect of radiation on the cell cycle by measuring BrdUrd incorporation to assess cells in S phase. We found that, in quiescent A431 cells, irradiation (4 Gy) resulted in an increased S-phase fraction within 2 h (Fig. 2B), which lasted for 24 h (data not shown), indicating that radiation stimulated G1/S-phase progression (5-22%). Similar results were obtained in UMSSC-1 cells (Supplementary Fig. S1). A brief, nontoxic exposure to EGFR and ERK activity inhibitors, erlotinib and U0126, respectively, blocked the progression of quiescent cells to S phase (Fig. 2B), supporting the involvement of EGFR-ERK signaling in S-phase progression in response to radiation. Conversely, EGFR stimulation in these cells increased the progression of cells to S phase in response to radiation (22-46%), confirming the role of EGFR activation in this phenomenon (Fig. 2B). These findings suggest that the increased radiosensitivity of quiescent cells compared with proliferating cells is driven by the EGFR-ERK signaling pathway.

**Effect of growth factor stimulation or inhibition on radiation sensitivity of quiescent and proliferating cells.** We then investigated the role of radiation-induced EGFR phosphorylation in radiation-induced cell death. As shown in Fig. 2B, EGFR preincubation potentiated quiescent cell transition from G1 to S phase; we therefore hypothesized that EGF preincubation would sensitize quiescent cells to radiation. We further hypothesized that treatment with a brief exposure to erlotinib (3 μmol/L) would block this transition and would therefore protect cells from radiation-induced clonogenic death. Indeed, we found that EGF pretreatment moderately sensitized A431 cells to radiation (enhancement ratio = 1.3 ± 0.01), and erlotinib pretreatment caused significant radioprotection (enhancement ratio = 0.54 ± 0.07; P < 0.002; Fig. 3A). We further sought to determine the role of radiation-induced ERK phosphorylation in radiation-induced cell death in quiescent cells. As shown in Fig. 2B, U0126 inhibited cell transition from G1 to S phase. We hypothesized that ERK inhibition, achieved using a brief nontoxic concentration of U0126, would...
protect cells from radiation-induced cell death. Indeed, we found that U0126 (10 μmol/L) protected quiescent cells from radiation-induced death (Fig. 3A). In contrast, neither EGF, erlotinib, nor U0126 significantly affected the radiosensitivity of proliferating cells (Fig. 3B). In quiescent cells, EGF pretreatment potentiated both EGFR and ERK activation in response to radiation, whereas erlotinib abrogated this activation (Fig. 3C). These signaling changes did not occur in proliferating cells (data not shown). Furthermore, U0126 completely blocked ERK activation in response to radiation after 1 h (Fig. 3D), confirming that EGFR-ERK activation is important in radiation-induced cell death. These findings suggest that radiation-induced EGFR phosphorylation and subsequent ERK phosphorylation drive irradiated quiescent cells into S phase, leading to cell death.

**Effects of exogenously expressed EGFR on radiation-induced clonogenic death in proliferating and quiescent cells.** Finally, we sought to explicitly assess the influence of proliferation status on radiosensitivity in CHO cells, which lack EGFR, and in CHO cells transfected so that they express EGFR. CHO cells are capable of expressing EGFR and have been used in studies of EGFR trafficking and function (17–21). Our experiments show that, in EGFR-expressing CHO cells, EGFR and ERK are phosphorylated on EGF stimulation (data not shown), confirming that this pathway functions appropriately in this system. In contrast to our findings in EGFR-expressing A431 cells, we found no difference between the radiation sensitivities of quiescent and proliferating wild-type CHO cells (enhancement ratio = 1.01 ± 0.01), which is consistent with a critical role for EGFR in radiation response (Fig. 4A). Furthermore, quiescent CHO cells ectopically expressing EGFR showed increased radiation sensitivity (enhancement ratio = 1.5 ± 0.2; P < 0.001) compared with proliferating cells (Fig. 4B). Finally, we confirmed that ectopic expression of EGFR in CHO cells resulted in EGFR activation in response to radiation “only” in quiescent cells and not in proliferating cells (Fig. 4C and D). These results confirm the role of EGFR activation in radiation-induced cell death in quiescent cells.

**Discussion**

In this study, we have shown that EGFR inhibition has a distinctly different effect on the radiosensitivity of quiescent and proliferating EGFR-driven cells in culture. Radiation-induced activation of EGFR in quiescent cells induces progression into S phase, slows DNA repair, and enhances radiation-induced cell death. The initial effect of EGFR inhibition is to block these processes and to cause radioprotection. However, in proliferating cells, radiation induces neither EGFR activation nor S-phase entry, and (short-term) EGFR inhibition has little effect on radiation-induced death. Collectively, our data show that cellular proliferation status profoundly affects EGFR and ERK activation in response to radiation, which significantly affects radiosensitivity. As tumors are composed of a heterogeneous mixture of both proliferating and quiescent cells, our findings support a more complicated model of tumor response to EGFR inhibition and radiation, in which the overall effect of EGFR inhibition may vary as a function of the number of quiescent versus proliferating cells.

Our findings in quiescent cells are consistent with prior reports that radiation stimulates EGFR activation in growth-arrested cells (9, 22). This radiation-induced EGFR activation induces progression to S phase, increasing radiosensitivity and cell death. However, our results differ in proliferating cells, wherein we found that radiation did not induce EGFR activation. This is in keeping with our previous work, in which we reported that, in proliferating cells, radiation appears to have little effect on already high levels of EGFR activation; instead, radiation actually decreases ERK activity in an ATM-dependent manner (10). It now appears that one cannot generalize that quiescent cells are less radiosensitive than proliferating cells (23); in fact, the opposite is found in EGFR-driven cells. These findings also suggest that the effects of radiation should also be evaluated in cells that are driven by similar tyrosine kinase proteins, including HER-2 and insulin-like growth factor-I-overexpressing cells.
Note that the conditions we used in our study differed from those that produce “potentially lethal damage repair,” in which cells that are held in plateau phase for 24 h after relatively high doses of radiation (10 Gy) show decreased sensitivity to radiation compared with those that are plated immediately after radiation (24, 25). We chose to use a lower dose of radiation (4 Gy) and to wait <4 h after radiation before processing cells to avoid confounding effects. Thus, our finding of increased radiation sensitivity in EGFR-expressing confluent cells is not in conflict with previous studies of potentially lethal damage repair.

Because we have observed defective repair capabilities of quiescent cells compared with proliferating cells, it appears that EGFR and ERK activation in quiescent cells may inappropriately drive the cells to S phase through a defective G2-S checkpoint, enhancing cellular radiosensitivity. This finding is consistent with the mechanism of action of chemoradiotherapy, such as gemcitabine or 5-fluorouracil, in combination with radiation, whereby inappropriate progression through S phase increases radiosensitivity (26–28). This model of increased cell killing following EGFR activation also resembles chemotherapy-induced cell death in that we previously reported that EGFR activation in response to chemotherapy is an indispensable event in subsequent cell death (29).

Our study helps to distinguish the direct, early effect of EGFR inhibition from the downstream effects of EGFR inhibition. The direct effect of EGFR inhibition can be mild to significant radioprotection, whereas downstream effects can induce accumulation in G2, leading to radiosensitization. As such, the study of activation of EGFR and ERK in response to radiation has yielded varying results, suggesting that this activation may depend on culture conditions, time of monitoring response, and dose of radiation. For instance, Harari and Huang found that, 24 h after EGFR inhibition, UMSCC-1 cells showed G2 arrest, decreased S-phase fraction, and enhanced radio-sensitivity (11). Tanaka and colleagues also found enhanced radiosensitivity in non-small cell lung cancer cells 24 h after EGFR inhibition (30). In our study, we irradiated cells 2 h after exposure to erlotinib, when EGFR was inhibited but before substantial cell cycle effects occurred; these conditions produced radioprotection.

The effects of radiation on ERK activity can also differ depending on direct versus indirect induction. As we and others (9, 31, 32) have found, ERK can be stimulated directly from EGFR activation in quiescent cells, whereas our previous work shows that ERK can be inhibited in response to radiation via MKP-1 activation.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
15. Hoffmann T, Hafner D, Ballo H, Bier H, Haas I, Haas I. Anti-
tumor activity of anti-epidermal growth factor receptor
monoclonal antibodies and cisplatin in ten human head
and neck squamous cell carcinoma lines. Anticancer
16. Chun PY, Feng FY, Scheurer AM, Davis MA, Lawrence
TS, Nyati MK. Synergistic effects of gemcitabine and
gefitinib in the treatment of head and neck carcinoma.
17. David T, Headinger JM, Gee P, Khan EM, Goldkorn T.
c-Blk-mediated ubiquitinylation is required for epider-
mal growth factor receptor exit from the early endo-
WY, Dikie I. Cbl-CIN85-endophilin complex mediates li-
gand-induced downregulation of EGF receptors. Nature
19. Boerner JL, Demory ML, Silva C, Parsons SJ. Phos-
phorylation of Y845 on the epidermal growth factor re-
ceptor mediates binding to the mitochondrial protein
cytochrome c oxidase subunit II. Mol Cell Biol 2004;
20. Clark S, Dunlop M. Modulation of phospholipase
A2 activity by epidermal growth factor (EGF) in
CHO cells transfected with human EGF receptor. Role
of receptor cytoplasmic subdomain. Biochem J 1991;
274:715–21.
boronated epidermal growth factor-dextran conju-
13:279–89.
22. Yacoub A, McKinstry R, Himman D, Chung T,
Dent P, Hagan MP. Epidermal growth factor and ion-
izing radiation up-regulate the DNA repair genes
XRCC1 and ERCC1 in DU145 and LNCaP prostate
carcinoma through MAPK signaling. Radiat Res
6th ed. Philadelphia: Lippincott Williams & Wilkins;
2006.
24. Weichselbaum RR, Little JB. Radioreistance in some
human tumor cells conferred in vitro by repair of poten-
25. Weichselbaum RR, Malcolm AW, Little JB. Fraction
size and the repair of potentially lethal radiation dam-
age in a human melanoma cell line. Possible implica-
26. Lawrence TS, Blackstock AW, McGinn C. The mech-
anism of action of radiosensitization of conventional
chemotherapeutic agents. Semin Radiat Oncol 2003;13:
13–21.
27. McGinn CJ, Miller EM, Lindstrom MJ, Kunugi KA,
Johnston PG, Kinsella TJ. The role of cell-cycle redistri-
bution in radiosensitization—implications regarding
the mechanism of fluorodeoxyuridine radiosensitiza-
28. Wilson GD, Bentzen SM, Harari PM. Biologic basis
for combining drugs with radiation. Semin Radiat Oncol
2006;16:2–9.
29. Feng FY, Varambally S, Tomlins SA, et al. Role of
epidermal growth factor receptor degradation in gem-
citabine-mediated cytotoxicity. Oncogene 2007;26:
3431–9.
30. Tanaka T, Munshi A, Brooks C, Liu J, Hobbs ML,
Meyn RE. Gefitinib radiosensitizes non-small cell lung
cancer cells by suppressing cellular DNA repair capaci-
ation-induced activation of multiple intracellular signal-
32. Lammering G, Hewit TH, Hawkins WT, et al. Epider-
mal growth factor receptor as a genetic therapy target
for carcinoma cell radiosensitization. J Natl Cancer Inst
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