Mechanisms of Enhanced Radiation Response following Epidermal Growth Factor Receptor Signaling Inhibition by Erlotinib (Tarceva)

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Abstract

Erlotinib (Tarceva) is an orally available HER1 (epidermal growth factor receptor, EGFR) tyrosine kinase inhibitor advancing through clinical trials for the treatment of a range of human malignancies. In this study, we examine the capacity of erlotinib to modulate radiation response and investigate specific mechanisms underlying these interactions in human tumor cell lines and xenografts. The impact of erlotinib on cell cycle kinetics was analyzed using flow cytometry, and the impact on apoptosis was evaluated using fluorescence-labeled pan-caspase inhibition and poly(ADP-ribose) polymerase cleavage. Radiation-induced EGFR autophosphorylation and Rad51 expression were examined by Western blot analysis. Radiation survival was analyzed using a clonogenic assay and assessment of in vivo tumor growth was done using a mouse xenograft model system. Microarray studies were carried out using 20 K human cDNA microarray and select genes were validated using quantitative reverse transcription-PCR (RT-PCR). Independently, erlotinib and radiation induce accumulation of tumor cells in G1 and G2-M phase, respectively, with a reduction of cells in S phase. When combined with radiation, erlotinib promotes a further reduction in S-phase fraction. Erlotinib enhances the induction of apoptosis, inhibits EGFR autophosphorylation and Rad51 expression following radiation exposure, and promotes an increase in radiosensitivity. Tumor xenograft studies confirm that systemic administration of erlotinib results in profound tumor growth inhibition when combined with radiation. cDNA microarray analysis assessing genes differentially regulated by erlotinib following radiation exposure identifies a diverse set of genes deriving from several functional classes. Validation is confirmed for several specific genes that may influence radiosensitization by erlotinib including Egr-1, CXCL1, and IL-1β. These results identify the capacity of erlotinib to enhance radiation response at several levels, including cell cycle arrest, apoptosis induction, accelerated cellular repopulation, and DNA damage repair. Preliminary microarray data suggests additional mechanisms underlying the complex interaction between EGFR signaling and radiation response. These data suggest that the erlotinib/radiation combination represents a strategy worthy of further examination in clinical trials. (Cancer Res 2005; 65(8): 3328-35)

Introduction

The epidermal growth factor receptor (EGFR) belongs to the ErbB family consisting of four closely related cell membrane receptors: EGFR (HER1 or ErbB1), ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4; ref. 1). Increased expression of EGFR has been observed in a wide spectrum of tumors, including non–small cell lung cancer (NSCLC) and squamous cell carcinoma (SCC) of the head and neck (2, 3). Activation of the EGFR signal transduction pathway has been shown to enhance cellular processes involved in tumor growth and progression, including the promotion of proliferation, angiogenesis, invasion, and metastasis (1). Increased expression of EGFR has been correlated with disease progression and poor overall clinical outcome (4, 5). Further, a positive correlation has been described between EGFR expression and tumor resistance to chemotheraphy and radiation therapy (6, 7).

A broad series of in vitro and in vivo studies have shown the potential value of targeting the EGFR in cancer treatment. Erlotinib (Tarceva, OSI-774) is an EGFR-selective tyrosine kinase inhibitor that blocks signal transduction pathways implicated in tumor cell proliferation, survival, and other host-dependent processes promoting cancer progression. Erlotinib inhibits the activity of purified EGFR tyrosine kinase and EGFR autophosphorylation in intact tumor cells, with IC50 values of 2 and 20 nmol/L, respectively (8). Erlotinib is under investigation in clinical trials for a variety of tumor sites, used both as monotherapy as well as in combination with chemotherapy and/or radiation (9–12).

A series of recently published studies show strong preclinical evidence regarding the capacity of EGFR inhibition to enhance the antitumor activity of ionizing radiation (13–16). The potential significance of the favorable interaction between EGFR inhibition and radiation recently realized a major clinical milestone with results from a phase III trial in advanced head-and-neck cancer patients. This international study showed a near doubling of median survival for patients treated with the EGFR inhibitor cetuximab over that achieved with radiation alone (17). There was a statistically significant improvement (log rank \( P = 0.02 \)) in locoregional disease control (8% at 2 years) and overall survival (13% at 3 years) favoring the cetuximab arm. These pivotal results will stimulate new clinical trials that incorporate EGFR inhibitors in combination with radiation for a variety of cancer types in which radiation plays a central treatment role.

This study examines the in vitro and in vivo capacity of the EGFR tyrosine kinase inhibitor erlotinib to modulate radiation response in human tumor cell lines and xenografts. The results suggest strong potential for mechanistic synergy between EGFR inhibition and radiation response at several levels, including cell cycle kinetics, apoptosis induction, and the targeting of accelerated cellular...
repopulation. The potential relationship between EGFR signaling and DNA damage repair is strengthened by new data regarding the inhibition of Rad51 expression by erlotinib. To gain further insight regarding the influence of EGFR signaling on radiation response, microarray studies were done to examine differential gene regulation. Several promising leads linking EGFR signaling and radiation response involving genes regulating cell structure, adhesion, apoptosis, and tumor angiogenesis are identified.

Materials and Methods

Reagents. Cell culture media were obtained from Life Technologies, Inc. (Gaithersburg, MD). Primary antibodies against EGFR and EGR-1 C-19 were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); pEGFR-1068 was obtained from Cell Signaling Technologies (Beverly, MA); proliferating cell nuclear antigen (PCNA) and Rad51 were obtained from Neomarker (Freemont, CA); and α-tubulin was obtained from Oncogene Research Products (Cambridge, MA). ECL+ chemiluminescence detection system was purchased from Amersham (Arlington Heights, IL). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Erlotinib was generously provided by OSI Pharmaceuticals (Melville, NY). All other chemicals were purchased from Sigma Chemical.

Cell lines. Human NSCLC (H226) and prostate cancer (DU145) cells were obtained from the American Type Culture Collection (Rockville, MD) and maintained in complete culture media consisting of RPMI (7.4) supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin. Human head-and-neck SCC cell lines (UM-SCC1 and UM-SCC6) were obtained from the Brown Laboratory (Arlington Heights, IL) and maintained as described by the Brown and Derisi Labs (available at http://fatigo.bioinfo.cnio.es/).

Cell cycle analysis. Cells were harvested and incubated with 1 µmol/L 5-FOA-galactose for 24 hours exposure to erlotinib, 24 hours after exposure to 6 Gy radiation, or the combination. Cells were harvested by trypsinization, washed with PBS, fixed, and stored at 4°C before DNA analysis. After removal of ethanol by centrifugation, the cell suspension was incubated with 10% fetal bovine serum and 1% penicillin and streptomycin. DNA distributions were analyzed by Modfit (Verity Software House, Inc., Topsham, ME) for the proportion of cells in sub-G1, G0, S, and G2-M phases of the cell cycle.

Apoptosis by fluorescence-labeled caspase inhibitors. Caspase activity was analyzed by fluorescence spectroscopy according to the manufacturer’s protocol (Chemicon International, Temecula, CA). Briefly, 24 hours after seeding, cells were exposed to erlotinib (1.0 µmol/L × 48 hours), X-ray therapy (XRT, 6 Gy), or the combination. Cells were harvested and resuspended to a final concentration of 2 × 10⁶ cells/mL. Three hundred microliters of this cell suspension were incubated with 1× fluorescence-labeled pan-caspase inhibitor FAM-VAD-FMK (18) at 37°C for 1 hour, washed, and resuspended in 320 µL PBS. A 100 µL aliquot of the cell suspension was transferred to a black 96-well plate in triplicate. Fluorescence was analyzed on a SpectraMax fluorescence plate reader at 550 nmol/L excitation and 600 nmol/L emission wavelengths.

Immunoblot analysis. Following treatment, cells were lysed with radioimmunoprecipitation assay buffer and sonicated in complete proteinase inhibitor cocktail (Roche, Indianapolis, IN) and sodium orthovanadate. Fifteen micrograms of protein extracts were mixed with SDS sample buffer and electrophoresed onto a 10% SDS-polyacrylamide gel and transferred onto nitrocellulose membranes (Amersham Pharmacia Biotech, Piscataway, NJ). Following blocking, membranes were incubated with specific primary antibodies, washed, and then incubated with horseradish peroxidase–linked secondary antibody (Amersham Pharmacia Biotech). The signals were visualized with the ECL+ detection system and autoradiography.

Enhanced Radiation Response following EGFR Inhibition

Radiation survival. Survival following radiation exposure was defined as the ability of the cells to maintain clonogenic capacity and form colonies. Briefly, after exposure to radiation, cells were trypsinized, counted, and seeded for colony formation in 35 mm dishes at 50 to 5,000 cells/dish. After incubation intervals of 14 to 21 days, colonies were stained with crystal violet and manually counted. Colonies consisting of 50 cells or more were scored, and five replicate dishes containing 10 to 150 colonies per dish were counted for each treatment. Experiments were done in duplicate.

Assay for tumor growth in athymic nude mice. In vivo studies were done in accord to institutional guidelines as described previously (19). Briefly, UM-SCC1 and H226 cells (∼1 × 10⁶) were injected s.c. into the flank area of athymic nude mice on day 0. Animal experiments included four treatment groups: control, radiation alone (2 Gy per fraction), erlotinib alone (0.8 mg/d), and radiation in combination with erlotinib. Erlotinib was administered by oral gavage once daily for 3 weeks. Radiation treatment was delivered twice a week for 3 weeks using custom mouse jigs designed to expose only the tumor bed to radiation.

Immunohistochemical determination of proliferating cell nuclear antigen and phosphorylated epidermal growth factor receptor. The expression of proliferative makers and activated EGFR were detected in histologic sections of H226 xenografts as described previously (19). Briefly, excised tumor specimens were fixed in 10% neutral-buffered formalin. After embedding in paraffin, 5 μm sections were cut and tissue sections were mounted. Sections were deparaffinized, rehydrated, and quenched endogenous peroxidase activity and blocking nonspecific binding sites, slides were incubated at 4°C overnight with 1:100 dilution of primary antibody directed against PCNA and phosphorylated EGFR (p-EGFR) followed by a 30-minute incubation in biotinylated secondary antibody. Slides were then incubated with streptavidin peroxidase and visualized using the 3,3′-diaminobenzidine chromogen (Lab Vision, Corp., Freemont, CA).

DNA microarray. DNA microarray analysis of gene expression was done as described by the Brown and Derisi Labs (available at http://www.microarrays.org). Cells exposed to radiation ± 24 hour pretreatment to erlotinib were solubilized and homogenized in TRIZol (Invitrogen, Carlsbad, CA). Total RNA was isolated according to the manufacturer’s instruction and integrity was tested. Once isolated, mRNA was used as a template for cDNA generation using reverse transcriptase. Inclusion of amino allyl-DUTP in the reverse transcriptase reaction allowed for subsequent fluorescent labeling of cDNA using monofunctional NHS ester dyes (as described at http://www.microarrays.org). In each experiment, fluorescent cDNA probes were prepared from an experimental mRNA sample (Cy5 labeled) and a control mRNA sample (Cy3 labeled) isolated from untreated cells. The labeled probes were then hybridized to 20 K human cDNA microarrays. Fluorescent images of hybridized microarrays were obtained using a GenePix 4000A microarray scanner (www.axon.com, Axon Instruments, Union City, CA). The Cy5/Cy3 ratio was collected and the data sets for each experiment were queried for genes that were differentially expressed in the drug-treated versus control cell lines (ratios >2.0 or <0.5; ref. 20). The data sets from individual analyses were then visualized using the TreeView Program. Identified genes were subsequently categorized using the web-based gene ontology program Fatigo (http://fatigo.bioinfo.cnio.es/).

Quantitative real-time PCR. To further validate microarray findings, we did quantitative real-time PCR using the SYBR green dye as previously described (21). Briefly, 1 µg total RNA isolated from each sample was reverse transcribed into first-strand cDNA. Threshold levels were set for each experiment during the exponential phase of the PCR reaction using the SDS version 1.7 software (Applied Biosystems, Foster City, CA). Quantity of DNA in each sample was calculated by interpolating its Ct value versus a standard curve of Ct values obtained from serially diluted cDNA from a mixture of all of the samples using Microsoft Excel. The calculated quantity of the target gene for each sample was then divided by the average calculated quantity of the housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and hydroxymethylbilane synthase (HMBS) corresponding to each sample to give a relative expression of the target gene.
gene for each sample. All oligonucleotide primers were synthesized by Integrated DNA Technologies (Coralville, IA). Primers for HMB5 and GAPDH were as described (22). Oligonucleotide primers for CXCL1, IL-1β, and Egr-1 are available upon request. All experiments were done in duplicate.

Statistics. The effects of erlotinib and/or radiation on growth inhibition in xenograft studies were assessed by multiple regression analysis using the PROC GLM procedure in SAS (version 8, SAS Institute, Inc., Cary, NC, 1999). Combination studies determining apoptosis induction were evaluated using Student’s t test with the resultant P representing a two-sided test of statistical significance.

Results

Cell cycle kinetics. The capacity of erlotinib to inhibit cell cycle progression and to modulate interaction with radiation was evaluated via flow cytometry. The effect of erlotinib on cell cycle phase distribution for the UM-SCC6 and H226 cell lines is summarized in Fig. 1. Erlotinib and ionizing radiation induced accumulation of cells in G1 and G2, respectively, and reduced the number of cells in S phase. When combined with radiation, erlotinib promoted a further reduction in the S-phase fraction. Although test results were reproducible and consistent, the trend of decreased S-phase fraction did not yield statistical significance (P = 0.1).

Erlotinib enhances radiation-induced apoptosis. We further evaluated whether mechanisms of interaction between erlotinib and radiation involve cell killing mediated by apoptosis in head-and-neck SCC and NSCLC cell lines. As shown in Fig. 2A, erlotinib (1 μmol/L) and radiation alone (6 Gy) induced a 10% to 25% and 25% to 50% increase in apoptosis, respectively, as determined by caspase activity. However, combined treatment with radiation and erlotinib resulted in an additive increase in apoptosis in H226 and UM-SCC1 cells and a supra-additive increase in apoptosis induction (P < 0.05) in UM-SCC6 cells. The enhancement of radiation-induced apoptosis by erlotinib was further confirmed using Western blot analysis to determine cleavage of the death substrate, poly(ADP-ribose) polymerase (PARP). As shown in Fig. 2B, 10 and 24 hours following treatment, erlotinib and radiation alone induced modest PARP cleavage. Further increase in PARP cleavage is shown when erlotinib is combined with radiation in UM-SCC1 cells. This enhancement in PARP cleavage with the erlotinib/radiation combination is even more pronounced in the UM-SCC6 cells (data not shown).

Erlotinib inhibits radiation-induced activation of epidermal growth factor receptor. Treatment with ionizing radiation can induce the EGFR proliferative pathway by the release of transforming growth factor-α and activation of the EGFR tyrosine kinase. This effect has been proposed to represent a central mechanism for accelerated cellular repopulation during radiation treatment (23). As depicted in Fig. 3, increased EGFR autophosphorylation was confirmed following radiation exposure (2 and 10 Gy) in three distinct cell lines (head and neck, lung, prostate). This radiation-induced activation of EGFR phosphorylation was profoundly inhibited by pretreatment exposure of tumor cells to 1 μmol/L erlotinib for 24 hours in culture.

Erlotinib attenuates radiation-induced expression of Rad51. The repair protein Rad51 represents a central component of homologous recombination during DNA repair (24, 25). Inhibition of Rad51 expression has been shown to correlate with increased radiation sensitivity (26). To examine the effect of erlotinib on Rad51 expression following radiation exposure, H226 and UM-SCC6 cells were exposed to radiation ± pretreatment with erlotinib. As depicted in Fig. 4, both cell lines show little to no detectable baseline Rad51 expression. An increase in Rad51 expression was shown following radiation exposure in a time-dependent manner (10 and 24 hours). This increase in Rad51 expression was confirmed following radiation exposure in a time-dependent manner (10 and 24 hours).
expression was attenuated significantly when cells were pre-treated with 1 μmol/L erlotinib for 24 hours.

**Erlotinib modulates radiosensitivity.** To examine the potential usefulness of combining erlotinib with radiation therapy in human carcinoma cell lines, experiments were conducted to evaluate the influence of erlotinib on clonogenic survival. Figure 5 depicts clonogenic survival curves for UM-SCC1 and H226 cell lines exposed to erlotinib before radiation exposure. These results show that treatment with erlotinib before radiation induced modest but consistent radiosensitization as manifested by a reduction in clonogenic survival compared with controls in UM-SCC1 at 3, 6, and 9 Gy (P < 0.01) and in H226 at 6 and 9 Gy (P < 0.05).

**Erlotinib augments in vivo tumor response of non–small cell lung cancer and squamous cell carcinoma xenografts to radiation.** Human NSCLC (H226) and SCC (UM-SCC6) cell lines were inoculated s.c. into female athymic mice and allowed to grow for 10 days before randomization into four groups. Ten days was the time interval required for xenografts to reach ~20 mm³ in volume. As shown in Fig. 6, treatment with radiation alone or erlotinib alone produced modest inhibition of tumor growth in both H226 and UM-SCC6 xenografts. When combined with radiation, erlotinib enhanced the tumor growth inhibition profile over the 55-day observation period.

**In vivo expression of proliferating cell nuclear antigen and phosphorylated epidermal growth factor receptor.** The expression of markers of tumor proliferation (PCNA) and activated EGFR (p-EGFR) were examined in H226 tumor xenografts. Immunohistochemical staining with PCNA showed the number of proliferating cells to be largest in the control group, intermediate in the groups receiving single-modality treatment with either radiation or erlotinib, and smallest in the combined treatment group. Immunostaining for p-EGFR showed similar activity in the control and radiation-treated groups, with marked inhibition in the combined erlotinib/radiation group (Fig. 7). Taken together, these results complement in vitro data that shows the capacity of erlotinib to modulate cellular proliferation, apoptosis, and EGFR signaling activation as shown in Figs. 1 to 3.

**Microarray analysis.** To identify a cohort of genes linking EGFR signaling with radiation response, we used a 20,000-element (20 K) cDNA microarray consisting of known, named genes as well as numerous expressed sequence tags (27). Initial experimentation was done on UM-SCC6 cells to determine the temporal relationship of gene expression following exposure to radiation. These preliminary
studies (data not shown) identified the largest cohort of differentially regulated genes to emerge \(~24\) hours after exposure to radiation. Subsequent array studies were, therefore, done on UM-SCC6 cells \(24\) hours after exposure to radiation \(+\) pretreatment with erlotinib. We identified a diverse set of differentially regulated genes (ratios \(>2\) or \(<0.5\)) involving 14 functional classes (Fig. 8). We validated these DNA microarray findings for a select cohort of genes that may influence the radiosensitization capacity of erlotinib, including Egr-1, CXCL1, and IL-1\(\beta\) (Fig. 9). These validation studies confirmed a potent radiation-induced enhancement of Egr-1, CXCL1, and IL-1\(\beta\) expression, which was inhibited by pretreatment exposure to erlotinib.

**Discussion**

This study characterizes the capacity of the EGFR tyrosine kinase inhibitor erlotinib to modulate radiation response in human carcinoma cell lines and xenografts. These results augment emerging preclinical data demonstrating a favorable antitumor interaction between EGFR inhibitory agents and radiation (13–16). In parallel with previous reports regarding the EGFR monoclonal antibody cetuximab (Erbitux, C225) and the tyrosine kinase inhibitor gefitinib (Iressa, ZD1839), the magnitude of radiosensitization with erlotinib seems magnified in the \textit{in vivo} setting (13, 19). The current data suggest potential explanations for this enhanced effect at the level of cell cycle kinetics, DNA damage repair, and radiation-induced activation of EGFR, which may contribute to inhibition of accelerated cellular repopulation. In addition, cells received single fraction radiation \textit{in vitro}, whereas multiple fractions of radiation were delivered \textit{in vivo}, thereby amplifying the potential influence of erlotinib on DNA damage repair.

The influence of erlotinib on the host microenvironment (through inhibition of tumor angiogenesis) may also contribute...
to enhanced \textit{in vivo} radiation response. The link between EGFR signaling and tumor angiogenesis has been previously described (14, 19, 28). The impact of EGFR inhibitors like erlotinib may ultimately decrease tumor hypoxia, thereby rendering cells more susceptible to the cytotoxic effects of radiation (29). In addition, preliminary cDNA microarray studies suggest several other factors involving the tumor microenvironment that are modulated by erlotinib, including cytokines and structural and cellular adhesion proteins.

Previous reports have suggested the capacity of EGFR inhibition to interfere with the activity or localization of DNA-protein kinase, which plays a central role in DNA double-strand-break repair (30). The current study further supports a relationship between EGFR signaling and DNA damage/repair by linking erlotinib with the DNA damage repair protein, Rad51. Rad51 represents a key protein in homologous recombination during DNA double-strand-break repair (24, 25). Attenuation of Rad51 expression has been shown to enhance radiation sensitivity (26) and Rad51 overexpression by tumor cells suggests that this represents a worthy molecular target for tumor radiosensitivity modulation (31). The protein expression data in Fig. 4 shows the capacity of erlotinib to attenuate radiation-induced expression of Rad51. Although a novel finding, similar interactions have been identified with other tyrosine kinase signaling pathways, including bcr-abl (25, 32) and insulin-like growth factor (33). We are further examining this potential mechanism of radiosensitization by erlotinib using RNA interference and immuno-cytochemistry.

Differential cell cycle phase sensitivity to the cytotoxic effects of radiation is well established, with S phase being more resistant and G2-M more sensitive to radiation (34). In the current study, independent exposure of cells to erlotinib or radiation elicits a characteristic G1 and G2-M phase arrest, respectively. Erlotinib exposure alone reduces the percentage of cells in the radiation-resistant S-phase fraction. When combined with single-fraction radiation, erlotinib precipitates a further decrease in the S-phase fraction. Depending on the duration of cell cycle arrest, subsequent radiation treatments might, therefore, be expected to encounter a higher percentage of cells in more radiation-sensitive phases of the cell cycle. This cell cycle kinetics interaction might explain the enhanced radiation response shown using \textit{in vivo} models, which used fractionated radiation regimens.

Cytotoxic therapies can trigger surviving tumor clonogens to divide more rapidly than before, a phenomenon termed accelerated repopulation. This proliferation of tumor cells during a radiation treatment course has been well defined as a factor that adversely impacts overall tumor response and ultimate local control (35). A proposed mechanism for accelerated cellular repopulation involves the capacity of ionizing radiation to activate EGFR, which is linked to several critical components of mitogenic and proliferative signaling (23). In the present study, we show the capacity of erlotinib to inhibit radiation-induced activation of EGFR signaling (Fig. 3), thereby providing a potential mechanism to enhanced \textit{in vivo} radiation response.
method to combat accelerated repopulation during fractionated radiation.

To further examine of potential EGFR/radiation interactions, preliminary microarray analysis of human tumor cells was done. These studies identified >100 genes that were differentially expressed (i.e., genes that were significantly up-regulated or down-regulated) following radiation, and subsequently normalized or reversed by erlotinib pretreatment. The identified genes represent several distinct functional classes involved in diverse oncogenic processes including cell structure, inflammation, adhesion, apoptosis, and tumor angiogenesis. Particular genes were selected, which may provide further insight regarding mechanistic synergy between EGFR signaling and radiation response including Egr-1 and the chemokines IL-1β and CXCL1, which have been linked to nuclear factor κB (NF-κB) activation.

Egr-1 encodes a zinc finger transcription factor that can be induced by a variety of stimuli, including growth factors, cytokines, and mitogens. A series of DNA-damaging agents have been reported to induce significant up-regulation of Egr-1 (36). Inhibition of Egr-1 expression has been shown to inhibit microvascular endothelial cell replication and migration, microtubule formation, vascular endothelial growth factor expression, and tumor angiogenesis (37, 38). Additionally, Egr-1 expression has been shown to increase EGFR expression during hypoxia (39). This study shows the capacity of erlotinib to attenuate Egr-1 expression following exposure to radiation. The potential impact of Egr-1 expression on tumor angiogenesis and overall EGFR expression is currently being explored in our laboratory.

Various interleukins and chemokines are induced and released following exposure to ionizing radiation. These molecules can participate either directly or indirectly in subsequent radiation response (40). We identified two cytokines, IL-1β and CXCL1, which may contribute to tumor growth and survival during radiation and may strengthen a link between EGFR signaling and the prosurvival signaling network, NF-κB (41, 42).

Several reports indicate that IL-1β induced by radiation can afford radioprotection (40) as well as stimulate tumor cell proliferation, angiogenesis, and invasion (43, 44). IL-1β exerts many biological effects by activating the transcription factor NF-κB, which in turn regulates the expression of a variety of inflammatory and oncogenic processes (45). The capacity of erlotinib to attenuate radiation-induced transcription of IL-1β may, therefore, decrease NF-κB DNA-binding activity. This link between ErbB signaling and NF-κB activity has recently been reported using the ErbB inhibitors trastuzumab and cetuximab (46, 47). Further studies to examine the interaction between EGFR inhibition and IL-1β transcription, and resulting impact on NF-κB activation and radiation response, are under way.

The CXC chemokine CXCL1, previously designated as melanoma growth stimulatory activity/growth related protein (MGSA/GRO), has recently been characterized as one of many chemokines involved in radiation response (48). CXCL1 has been shown to play an important role in tumorigenesis and angiogenesis and its overexpression has been associated with tumor progression (49). Increased expression of CXCL1 has been attributed to constitutive activation of NF-κB through mitogen-activated protein kinase (MAPK) signaling (49). Therefore, the capacity of erlotinib to inhibit MAPK signaling and to influence NF-κB activation may reflect a mechanistic interaction linking EGFR signaling with CXCL1 expression.

Figure 9. Microarray validation of selected genes differentially regulated by erlotinib (1.0 μmol/L × 24 hours) followed by radiation (6 Gy) in UM-SCC6 using quantitative SYBR green RT-PCR (A) and Western blot analysis (B). RT-PCR was done on each sample in duplicate and the ratio was calculated relative to the housekeeping genes HMBS and GAPDH.

Conclusion

These preclinical results identify potential cellular mechanisms whereby EGFR signaling inhibition can enhance tumor radiation response. The first phase III clinical trial to examine the combination of EGFR inhibitor plus radiation (head-and-neck cancer) indicates favorable outcome with increased patient survival over that achieved with radiation alone (17). The newly reported phase III clinical trial confirming survival extension in refractory NSCLC patients receiving erlotinib (50) also suggests opportunities to explore combination approaches in lung cancer, where radiation plays a central treatment role. Improved understanding of molecular interactions between EGFR signaling inhibition and radiation should assist in selecting susceptible tumors for this combined therapy approach, as well as suggest optimal dose, duration, and sequence strategies for clinical trial evaluation.

Acknowledgments

Received 10/6/2004; revised 1/18/2005; accepted 2/1/2005.

Grant support: NIH T-32 Physician Scientist Training grant CA 0096-14 at University of Wisconsin (P. Chinnaiyan). P.M. Harari holds a research agreement with Genentech, which provided partial support for preclinical studies with erlotinib.

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We thank Genentech (San Francisco, CA) for generous provision of erlotinib for experimental studies.
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