Epidermal Growth Factor Receptor Autocrine Signaling in RIE-1 Cells Transformed by the Ras Oncogene Enhances Radiation Resistance

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ABSTRACT

Oncogenic forms of the small GTPase Ras increase the resistance of cells to killing by ionizing radiation (IR). Although not all of the signaling pathways for radioresistance are well defined, it is now clear that Ras-dependent signaling pathways involved in radioresistance include those mediated by phosphatidylinositol 3’-kinase (PI3-K) and Raf. Nevertheless, PI3-K and Raf together are not sufficient to reconstitute all of the resistance conferred by Ras, indicating that other effectors must also contribute. We show here that Ras-driven autocrine signaling through the epidermal growth factor receptor (EGFR) also contributes to radioresistance in Ras-transformed cells. Conditioned media (CM) collected from RIE-1 rat intestinal epithelial cells expressing oncogenic Ras increased the survival of irradiated cells. Ras-CM contains elevated levels of the EGFR ligand transforming growth factor alpha (TGF-α). Both Ras-CM and TGF-α stimulated EGFR phosphorylation, and exogenous TGF-α mimicked the effects of Ras-CM to increase radioresistance. Blocking EGFR signaling with the EGFR/HER-2 kinase inhibitor (Ki) GW572016 decreased the postradiation survival of irradiated Ras-transformed cells and normal cells but had no effect on the survival of unirradiated cells. Ras-CM and TGF-α also increase PI3-K activity downstream of the EGFR and increase postradiation survival, both of which are abrogated by GW572016. Thus, Ras utilizes autocrine signaling through EGFR to increase radioresistance, and the EGFR Ki GW572016 acts as a radiosensitizer. The observation that Ras-transformed cells can be sensitized to killing by ionizing radiation with GW572016 demonstrates that EGFR KIs could potentially be used to radiosensitize tumors in which radioresistance is dependent on Ras-driven autocrine signaling through EGFR.

INTRODUCTION

Activating Ras mutations are found in 30% of all cancers, and activation of signaling molecules both upstream and downstream of Ras is implicated in up to 90% (1). Cell lines transformed by Ras display many of the properties of cancer cells including increased growth rate, altered adhesion, and increased invasiveness and motility (2, 3). Although increased resistance to killing by IR1 is not always associated with transformation, Ras activation both in cell lines derived from normal tissues and in cell lines derived from tumors leads to enhanced radioresistance (4–10). Conversely, blocking Ras activity has been shown to increase radioresistance (9, 11–14). Because a third of all cancers are currently treated with radiotherapy, and because the sensitivity of cells to killing by IR is an important determinant of the probability of cure (15, 16), finding out how Ras can cause this radioresistant phenotype and developing ways to stop Ras-induced radioresistance would benefit many patients. We have set out to determine how Ras signaling enhances radioresistance.

Key components of signaling in normal epithelial cells include the Ras controlled signaling cascades and autocrine signaling, which involves the release and reception of growth factors. When growth factors engage receptor tyrosine kinases (RTKs), the RTKs become active through dimerization and phosphorylation of tyrosine residues on their cytosolic regions. Active RTKs then recruit and activate downstream signaling molecules, such as guanine nucleotide exchange factors for Ras. Guanine nucleotide exchange factors catalyze the exchange of GDP for GTP such that WT Ras becomes transiently GTP-bound and active. EGFR is one prominent RTK that transiently activates Ras, allowing Ras to signal through an array of downstream signaling pathways. Activated Ras up-regulates the PI3-K > Akt signaling pathway and the Raf > MEK > ERK signaling pathway in many cell types. Ras also signals through stress response signaling pathways involving p38 and c-Jun-NH2-terminal kinase mitogen-activated protein kinases, as well as through less well-defined pathways including the Raf GTP exchange factor RafGDS and the Rab exchange factor Rin1 (17–21). EGFR itself can also activate PI3-K and other signaling pathways independently of Ras (22). The activity of different subsets of these and other downstream pathways may lead to different phenotypic changes. Thus, one subset of signaling pathways may lead to increased growth rate, whereas another subset of signaling pathways may enhance resistance to IR. These ultimate phenotypic changes due to Ras signaling are dependent not only on signaling directly downstream of Ras but also on altered autocrine (cell-cell) signaling. Whereas Ras activity has been known to influence autocrine signaling for some time, it is still unclear which subsets of Ras signaling pathways are responsible.

Oncogenic Ras differs from WT Ras in that it is constitutively GTP-bound and thus is constitutively activated independently of RTK signaling. Oncogenic Ras utilizes autocrine signaling to mediate many of its oncogenic properties. Ras transformation of the epithelial cell lines RIE-1, IEC-6, and MCF-10A depends on Ras amplification of autocrine signaling (23), whereas Ras transformation of fibroblasts is less dependent on autocrine signaling. Increased expression of TGF-α has been associated with Ras transformation in various systems (24, 25), and other EGFR ligands including amphiregulin, HB-EGF, and β-cellulin may play a role as well (26). Activated Ras can thus amplify dysregulated autocrine signaling through EGFR to increase signaling to PI3-K and other EGFR effectors to mediate transformation. Thus, activated Ras may be responsible for the overstimulation of EGFR associated with some tumors. For example, TGF-α is overexpressed in the majority of breast, colorectal, and head and neck tumors, but the role of Ras activity in these situations is unknown (25, 27, 28). Other EGFR ligands are also found at higher levels in tumors than in normal tissues (28, 29). In addition, higher levels of EGFR ligands including TGF-α, epiregulin, and HB-EGF correlate with worse prognosis in bladder cancer and in head and neck squamous carcinoma (30–33). Overexpression of EGFR itself has been seen in many types of cancer, including breast, glioma, ovarian, and head and neck carcinoma, and correlates with aggressive tumor growth and...
poor prognosis in most (31, 34–38). Stimulation of EGFR by TGF-α overexpression has also been associated with poor prognosis (31, 37, 39, 40). This worse prognosis may be correlated with the enhanced radioresistance associated with stimulation of EGFR by autocrine signaling. For example, stimulation of EGFR activity by ligands has been associated with enhanced radioresistance in mammary carcinoma cells (41, 42). Blocking EGFR with monoclonal anti-EGFR antibodies, dominant-negative EGFR, or an EGFR KI has been shown to radiosensitize cancer cell lines (43–45). The anti-EGFR antibody C225 has shown promise in enhancing radiotherapy in humans, as described in several recent reviews (46–48). These methods predominantly block EGFR itself. Other EGFR family members, such as HER2, a heterodimerization partner of EGFR and other EGFR family members, also play a role in radioresistance (48, 49). A KI that can block the activity of both HER2 and EGFR, such as GW572016, formerly known as GSK572016 (50), would be advantageous in decreasing radioresistances of tumor cells with up-regulated HER2 activity as well.

While studying how Ras can induce radioresistance in our rat intestinal epithelial (RIE-1) model system, we showed that both PI3-K and Raf signaling play a role in radioresistance (51). However, RIE-1 cells transformed simultaneously with both PI3-K and Raf failed to recapitulate the level of radioresistance observed in Ras-transformed cells. Thus, we have sought to identify additional ways that Ras may increase radioresistance. Ras proteins mediate many of their actions through recruitment of Raf-1 to the cell surface, but activated Raf is insufficient to induce transformation of RIE-1 cells (23). Ras-expressing, but not Raf-expressing, RIE-1 cells transcribe higher levels of autocrine factors including insulin, hepatocyte growth factor, and the EGFR ligands, TGF-α, HB-EGF, and amphiregulin (52). This Ras-induced increase in EGFR ligands is required for Ras-mediated transformation of RIE-1 cells (23, 53). Blocking Ras activity in RIE-1 cells leads to a reduction in TGF-α and amphiregulin expression (53). Thus, Ras-transformed cells up-regulate signaling through the EGFR, and because higher levels of EGFR activity have been associated with radioresistance, we hypothesized that Ras-transformed cells may increase EGFR activity to cause part of the radioresistant phenotype seen in many Ras-transformed cells.

To determine whether Ras mediates radioresistance through autocrine signaling, we collected culture medium conditioned by both normal and Ras-transformed RIE-1 cells and treated normal RIE-1 cells with this medium prior to IR. We observed that Ras-CM enhanced radioresistance and stimulated phosphorylation of the EGFR. Pretreatment of cells with TGF-α, which we have shown previously to be present at higher levels in CM from Ras-transformed RIE-1 cells as compared with normal RIE-1 cells, also enhanced radioresistance and stimulated phosphorylation of EGFR. Inhibition of EGFR led to radiosensitization of both normal and Ras-transformed RIE-1 cells and also of cells stimulated with Ras-CM or TGF-α. We also examined signaling downstream of EGFR in cells treated with the EGFR KI and observed decreased PI3-K and Raf activity. We thus conclude that Ras mediates radioresistance in part by alteration of autocrine signaling and that signaling involves stimulation of the EGFR.

MATERIALS AND METHODS

Molecular Constructs. The pBABEpuro vector has been described previously (54). pBABE-H-ras(12V) was a gift of Aidan McFall and Channing J. Der [University of North Carolina, Chapel Hill, NC (55, 56)]. The constitutively active form of Raf-1(122W) has been described previously (57) and was transferred to pBABE (56).
30 min before lysis, cells were either fed fresh medium, placed in CM or treated with 20 ng/ml TGF-α and/or GW572016, or the vehicle was replaced if the culture medium was changed for CM treatment. Cells were lysed in Triton X-100 lysis buffer [50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1% (v/v) Triton X-100, 10% (v/v) glycerol, 1 μM sodium orthovanadate, 10 μM p-nitrophenyl phosphate, 20 μM β-glycerophosphate, 0.5 μM Pefabloc, 5 μg/μl leupeptin, and 10 μg/ml aprotinin]. Protein concentration was determined as described for immunoprecipitation. Twenty μg of protein for each sample were separated by SDS-PAGE on 10% SDS, 15% acrylamide low cross-linker gels (60). Separated proteins were electroblotted onto polyvinylidene difluoride membranes and blocked in TBST containing 5% nonfat powdered milk. Primary and secondary antibodies described below were added sequentially. After washing with TBST, the blots were developed using SuperSignal chemiluminescent substrate (Pierce, Rockford, IL).

Antibodies. Primary antibodies were all rabbit polyclonal antibodies (diluted 1:1,000 in Tris-buffered saline/Tween 20% BSA): anti-P-Akt (specific for Akt phosphorylated at serine 473); anti-Akt (Cell Signaling, Beverly, MA); and anti-ERK (sc-94; Santa Cruz Biotechnology). For all primary antibodies, the secondary antibody used was horseradish peroxidase-conjugated antirabbit IgG (Amersham, Arlington Heights, IL) diluted 1:30,000 in TBST.

RESULTS

TGF-α and TGF-α-Containing CM from Ras-Transformed Cells (Ras-CM) Increase Postirradiation Survival of Normal RIE-1 Cells. Ras transformation of RIE-1 cells leads to increased radioresistance, and RIE-Ras cells are characterized by increased production of autocrine growth factors, including the EGFR ligands TGF-α, amphiregulin (AR), and HB-EGF. Thus, we asked whether autocrine growth factor production induced by Ras transformation contributes to Ras-mediated radioresistance. Cells producing autocrine growth factors release those growth factors to “condition” the cell growth medium (CM). We have shown previously in RIE-1 cells that application to normal cells of CM from Ras-transformed cells (Ras-CM), but not of CM from normal cells (vector-CM), leads to a transformed phenotype (61). We collected Ras-CM that induces a transformed morphology in normal RIE-1 cells, whereas vector-CM does not alter cell morphology (data not shown). We then applied the CM to sparsely plated normal RIE-1 cells 30 min before irradiation. We observed that Ras-CM enhances survival of normal RIE-1 cells (Fig. 1A), indicating that Ras transformation can lead to secretion of autocrine factors that enhance radioresistance. Because previous work showed that Ras transformation of RIE-1 cells leads to increased production of several autocrine factors that are ligands for EGFR family members and that TGF-α was the most elevated over normal cells, we decided to test whether TGF-α could alter the radiation response of RIE-1 cells. When 10 ng/ml TGF-α was applied to normal RIE-1 cells, we observed a 1.6-fold enhancement of survival (Fig. 1B). We observed similar responses with a concentration as low as 1 ng/ml, but the effect of TGF-α falls off at the highest dose (50 ng/ml). We also tested whether TGF-α could alter the survival of RIE-Ras or RIE-Raf cells and observed that TGF-α enhances the radioresistance of these cells as well, suggesting that the effect of autocrine activity on radiation response is not already maximized (Fig. 1C) under these conditions. The autocrine component of Ras-mediated radioresistance may not be as readily measured by these clonogenic survival experiments in which cells are plated sparsely, but it is likely to have a greater effect in vivo, where many cells are in close proximity. In vivo, the elaborated autocrine factors are concentrated and are not diluted into a large volume of culture medium. In addition, surface-bound autocrine ligands can be presented efficiently to neighboring cells only under conditions of high cell density. Our data indicate that Ras-CM contains autocrine factors that enhance radioresistance of RIE-1 cells even under conditions of low cell density and that one known component of this CM, TGF-α, enhances radioresistance of both normal and transformed cells.

TGF-α-Containing Ras-CM Increases Phosphorylation of EGFR. Because activation of EGFR has been linked to radioresistance in multiple studies, and Ras-CM contains EGFR ligands, we...
reasoned that the mechanism by which Ras-CM enhances radioresistance was through activation of EGFR family members. The tyrosine residues of EGFR become phosphorylated when EGFR engages ligand, dimerizes, and transphosphorylates its dimerization partner. Tyrosine phosphorylation status of EGFR is routinely used as an indication of EGFR kinase activity. To determine whether there was EGF-like activity in the Ras-CM, we treated normal RIE-1 cells with fresh medium, CM, or TGF-α 30 min before lysis; performed EGFR immunoprecipitation; and then blotted for phosphotyrosine. As shown in Fig. 2, we detected an increase above the basal tyrosine phosphorylation in normal RIE-1 cells treated with either Ras-CM or TGF-α and to a much lesser extent in those treated with Raf-CM. Vector-CM stimulates phosphorylation of EGFR less well than fresh media because fresh media contains fresh serum and the growth factors contained in that serum, whereas the serum present in vector-CM is depleted of some growth factors by the cells that conditioned the media. The observation that Ras-CM and TGF-α similarly enhance EGFR phosphorylation supports our hypothesis that Ras-CM stimulates EGFR to enhance radioresistance.

The EGFR KI Blocks Phosphorylation of EGFR and Decreases Survival of RIE-1 Cells. To confirm that autocrine activation of the EGFR was responsible for the Ras-induced radioresistance, we evaluated the effect of an EGFR tyrosine KI, GW572016 blocks the kinase activity of EGFR and HER2 and thus should block TGF-α and other ligands from stimulating signaling cascades dependent on EGFR (62). Thus, we treated RIE-1 cells with the EGFR KI and then added CM or TGF-α. We observed that the KI completely blocked phosphorylation of EGFR both in cells treated with CM and in cells treated with TGF-α (Fig. 3A). We also observed that as little as 1 μg/ml of the EGFR KI resulted in complete inhibition of EGFR tyrosine phosphorylation (data not shown). Thus, the EGFR KI blocks EGFR function. When we similarly treated sparsely plated RIE-1 cells with either CM or TGF-α in the presence of the KI and then irradiated the cells, we observed that the KI blocked both the enhanced radioresistance seen with CM and the enhanced radioresistance seen with TGF-α (Fig. 3B). Thus, Ras transformation can lead to both signaling downstream of Ras to enhance radioresistance and secretion of EGFR ligands that enhance radioresistance. The EGFR KI additionally lowered the basal radioresistance seen in cells treated with CM or TGF-α (Fig. 3C), demonstrating that EGFR and HER2 are generally important for radioresistance and that Ras-transformed cells can be radiosensitized by EGFR inhibition.

Both Ras-CM and TGF-α Stimulate PI3-K Activity That Is Blocked by the EGFR KI. Ligand-bound EGFR activates multiple signaling pathways including Ras. In turn, Ras signaling branches through multiple signaling cascades involving PI3-K > Akt, Raf > MEK > ERK, and Raf > novel unknown effectors and other Ras-dependent signaling cascades. In addition, EGFR can activate...
PI3-K independently of Ras, as well as activate other signaling cascades, such as signal transducers and activators of transcription (STATs). Activation of either Ras or PI3-K has been shown to result in enhanced radioresistance of RIE-1 cells (51) and of other cell types as well (63). We hypothesized that Ras-CM may stimulate EGFR, which would activate Ras or PI3-K signaling cascades to enhance radioresistance. Therefore, we examined whether CM or TGF-α would result in activation of PI3-K signaling by using Akt phosphorylation status as a readout for PI3-K activity. We observed that treatment of cells with fresh medium, Ras-CM, Raf-CM, or TGF-α resulted in increased P-Akt levels, whereas untreated, starved cells did not display phosphorylated Akt (Fig. 4A). Fresh medium can stimulate phosphorylation of Akt due to the growth factors in serum. CM is collected from confluent cells, so the media are depleted of growth factors and nutrients. Thus, both vector-CM and Raf-CM result in less phosphorylation of Akt than fresh media because CM has been depleted of growth factors and because RIE-Raf and RIE-vector cells elaborate only low levels of EGFR ligands. Treatment with the EGFR KI blocks the Akt phosphorylation seen in all conditions, indicating that EGFR ligands are responsible for all of the detected PI3-K activity seen in the presence of CM. As with P-Akt, we also observed that fresh medium, Ras-CM, and TGF-α lead to increased levels of P-ERK, whereas Raf-CM and vector-CM fail to stimulate ERK phosphorylation (Fig. 4B). Again, this signaling can be blocked with the EGFR KI, indicating that Raf activity is dependent on EGFR stimulation by ligands in the CM. Thus, Ras-transformed cells can produce growth factors that stimulate EGFR and lead to Raf and PI3-K signaling, and this signaling may be a major player in the Ras-mediated radioresistance associated with autocrine signaling.

DISCUSSION

Oncogenic Ras Induces Radioresistance in Part by Activating EGFR Through Up-Regulation of Autocrine Signaling. Previously, we and others have shown that Ras transformation increases radioresistance and that this radioresistance is partially dependent on specific signaling pathways downstream of Ras (4, 51, 63, 64). These signaling pathways include Ras > PI3-K and Ras > Raf, but not the Raf effector MEK. However, experiments with stable cell lines expressing active forms of either PI3-K or Raf or both PI3-K and Raf show that a combination of these pathways is insufficient for the full radioresistance seen with active Ras. Thus, Ras uses additional signaling pathways for complete radioresistance. In a search for additional signaling pathways that Ras may use to increase radioresistance, we examined Ras-dependent autocrine signaling. Ras-transformed RIE-1 epithelial cells have been shown to up-regulate the production of EGFR ligands, particularly TGF-α (23, 52, 53). Ras has also been demonstrated to increase levels of TGF-α in other cell types (24, 25). We have now shown here that Ras-transformed cells use autocrine signaling through the EGFR to make RIE-1 epithelial cells resistant to radiation. We have shown here that the EGFR ligand, TGF-α, which we and others have previously demonstrated is up-regulated in RIE-1 cells by H-ras activity (23, 52, 53), is itself able to enhance radioresistance, suggesting that this is the component in Ras-CM that is responsible. Many tumors, some of which have activated Ras, have been demonstrated to elaborate higher levels of TGF-α (65, 66). The increased production of this and other EGF-like ligands may be partially responsible for the increased radioresistance seen in many tumors (25, 27, 28, 32). Others have shown that EGFR overexpression is positively correlated with radioresistance in murine carcinomas (67, 68) and that stimulation of EGFR by epidermal growth factor is associated with radioresistance in A431 and MCF-7 cells (41, 42). Now we have shown that Ras-induced autocrine factors can activate EGFR to enhance radioresistance. This is the first demonstration, to our knowledge, that oncogenic Ras can increase radioresistance through an autocrine signaling pathway.

Abrogation of EGFR Signaling by GW572016 Causes Radiosensitization. Like Ras, EGFR has long been considered to play an important role in radiation response of epithelial tumors. Clinically, overexpression of EGFR in tumors correlates with poorer response to radiotherapy (69), and increasing levels of EGFR correlate with radioresistance in head and neck cancer cell lines (70). Indeed, the EGFR is an attractive target for radiosensitization. Preclinical studies have shown effective radiosensitization using either anti-EGFR monoclonal antibodies (C225) or small molecule tyrosine KIs (ZD1839/Iressa, CI-1033, and GW572016) in both in vitro and in vivo models (45, 71–75). Based on preclinical efficacy, several Phase I/II trials testing the toxicity and efficacy of combining EGFR inhibitors with radiotherapy are under way or completed, and the results of a completed Phase III randomized trial in head and neck cancer are pending (reviewed in Ref. 48). However, considerable doubt remains as to the most appropriate selection of patients for enrollment on these trials because the mechanism of radiosensitization is not yet clearly defined. The EGFR inhibitors have pleiotropic tumor effects when used alone or combined with radiotherapy, including pronounced antiangiogenic effects [accompanied by decrease in EGFR-mediated vascular endothelial growth factor expression, perturbation of DNA repair, cell cycle alterations, and enhancement of radiation-induced apoptosis (47, 76)]. We have now shown that EGFR inhibitors exert yet another effect on radiation response by inhibiting a resistance-inducing Ras-mediated autocrine loop. Whereas the effect of EGFR KI GW572016 is more pronounced in EGFR-driven cell lines than in our RIE-1 cells (73), amplification of modest radiosensitizing effects with fractionated regimens in the clinical setting may result in meaningful differences in tumor control. Thus, should patients be selected for trials based on the level of EGFR expression or activation (as an indicator that the tumor pathogenesis involves EGFR), the level of vascular endothelial growth factor expression, Ras mutation, or other
normal cells that have been made radioresistant by oncogene-driven carcinoma cells (79). In addition, this EGFR KI has been shown to efficacy demonstrated by EGFR KIs of EGFR ligands. An interesting potential explanation for the greater sensitizers not only in tumors with EGFR overexpression but also in specifically engage EGFR to activate signaling cascades that enhance further demonstrates that the autocrine component of Ras-mediated radioresistance in this model. In addition, EGFR itself is activated by IR (77, 78), and thus, the EGFR KI blocks radiation-induced EGFR activation and the component of radioresistance induced by that activation. Finally, radiation-induced release of TGF-α has also been shown to enhance radioresistance in mammary carcinoma cells (79). In addition, this EGFR KI has been shown to decrease some of the transformed properties of Ras-driven tumors such as increased growth rate (62).

We have demonstrated here that GW572016 can also radiosensitize normal cells that have been made radioresistant by oncogene-driven paracrine signaling. The observation that this EGFR KI so specifically blocks the enhanced radioresistance seen with Ras-CM and TGF-α further demonstrates that the autocrine component of Ras-mediated radioresistance depends in part on activation of and signaling through EGFR and that Ras induces the elaboration of growth factors that specifically engage EGFR to activate signaling cascades that enhance survival. Our data suggest that EGFR KI may be effective as radiosensitizers not only in tumors with EGFR overexpression but also in tumors with activated Ras because Ras activity increases production of EGFR ligands. An interesting potential explanation for the greater efficacy demonstrated by EGFR KIs in vivo than in vitro, in addition to their effect on inhibition of angiogenesis, may be that autocrine survival factors, such as TGF-α, are more concentrated and effectively presented in tissue models than in a sparsely plated tissue culture experiment.

The observation that Raf-CM could moderately enhance radioresistance is in agreement with previous data showing that an active form of Raf leads to radioresistance and that an antisense form of raf can cause radiosensitization (51, 80). Raf-CM contains only low levels of TGF-α, amphiregulin, and HB-EGF mRNAs (52), but it mildly increased EGFR phosphorylation. We observed that the EGFR KI blocks the radioresistance seen with Raf-CM. Thus, we conclude that Raf-CM mediates moderate radioresistance via an EGFR autocrine loop through other EGFR family ligands, such as HER-2, which is also inhibited by GW572016 (50, 62, 73). It would be interesting to identify this ligand. Nonetheless, the effect of the Raf-elaborated ligands is substantially less than that of the CM-eliminated by Ras-transformed cells, and Ras obviously utilizes additional Raf-independent autocrine signaling to enhance radioresistance.

Cross-Talk Between a TGF-α Autocrine Loop and Direct Activation of PI3-K by Ras. How does autocrine signaling through the EGFR enhance radioresistance? PI3-K is an obvious candidate as a downstream mediator of EGFR-induced radioresistance because PI3-K is both necessary for Ras-mediated radioresistance and important for cell survival after IR in general (51, 63, 81). Multiple studies have examined Akt phosphorylation status as a readout for PI3-K activity in tumors with EGFR overexpression, and loss of Akt phosphorylation has been used as a measure of the effectiveness of EGFR inhibition (62, 69, 82). Akt itself is a mediator of radioresistance. 6

We have shown here that both Ras-CM and TGF-α lead to increased levels of both P-Akt and P-ERK. Thus, activated Ras can stimulate PI3-K and ERK activity both directly and through autocrine signaling to EGFR. We have also shown that GW572016 blocks the ability of CM and TGF-α to stimulate signaling through PI3-K and to ERK. The fact that we could block phosphorylation of Akt and ERK with the EGFR KI shows that P-Akt and P-ERK levels are increased by signaling through EGFR and not through other receptors that are stimulated by non-EGFR family ligands in Ras-CM. It is presently unclear whether the EGFR signaling to activate PI3-K goes through Ras or is independent of Ras.

The autocrine loop in Ras-transformed cells might increase radioresistance by further up-regulating either the signaling of endogenous WT Ras, EGFR-dependent but Ras-independent signaling downstream of PI3-K, or additional EGFR-dependent pathways such as the STAT pathway. Autocrine signaling from Ras may also lead to activity of EGFR-independent signaling pathways that mediate radioresistance. In summary, activated Ras leads to EGFR activity, which, in turn, activates Ras pathways in a positive feedback mechanism for radioresistance. Overexpression of EGFR family members could also lead to this positive feedback mechanism for radioresistance. All of these positive feedback pathways work together to mediate radioresistance in tumors, and blocking autocrine signaling with EGFR KIs such as GW572016 may enhance the effectiveness of radiotherapy.

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REFERENCES


6 A. Gupta, personal communication.


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