EGFR-Mediated Chromatin Condensation Protects KRAS-Mutant Cancer Cells against Ionizing Radiation

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Abstract
Therapeutics that target the epidermal growth factor receptor (EGFR) can enhance the cytotoxic effects of ionizing radiation (IR). However, predictive genomic biomarkers of this radiosensitization have remained elusive. By screening 40 non–small cell lung cancer cell (NSCLC) lines, we established a surprising positive correlation between the presence of a KRAS mutation and radiosensitization by the EGFR inhibitors erlotinib and cetuximab. EGFR signaling in KRAS-mutant NSCLC cells promotes chromatin condensation in vitro and in vivo, thereby restricting the number of DNA double-strand breaks (DSB) produced by a given dose of IR. Chromatin condensation in interphase cells is characterized by an unexpected mitosis-like colocalization of serine 10 phosphorylation and lysine 9 trimethylation on histone H3. Aurora B promotes this process in a manner that is codependent upon EGFR and protein kinase Cα (PKCα). PKCα, in addition to MEK/ERK signaling, is required for the suppression of DSB-inducible premature senescence by EGFR. Blockade of autophagy results in a mutant KRAS-dependent senescence-to-apoptosis switch in cancer cells treated with IR and erlotinib. In conclusion, we identify EGFR as a molecular target to overcome a novel mechanism of radioresistance in KRAS-mutant tumor cells, which stands in contrast to the unresponsiveness of KRAS-mutant cancers to EGFR-directed agents in monotherapy. Our findings may reposition EGFR-targeted agents for combination with DSB-inducing therapies in KRAS-mutant NSCLC.

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Introduction
Exposure of cellular DNA to ionizing radiation (IR) generates various types of damage (1). A dose of 1 Gy produces 20 to 40 DNA double-strand breaks (DSB) in a mammalian cell (1, 2). Unrepaired or misrepaired DSBs are the principal type of damage that may result in lethal chromosomal aberrations and cell death, or radiobiologically termed “cell inactivation,” within 1 to 3 division cycles (reviewed in ref. 3). Molecular targeted anticancer agents have been tested extensively preclinically, and increasingly so in the clinic, to enhance the cytotoxic effects of IR. Preclinical data suggest that radiosensitization is frequently achieved by interfering with DSB repair, thereby increasing the levels of residual, unrepaired DSB (4–6). Unrepaired DSB may induce cellular senescence or apoptosis, but the latter outcome is generally more desirable as senescent cells remain viable and can even escape senescence (7, 8).

Non–small cell lung cancer (NSCLC) is difficult to control locally by IR due to the typically large tumor size at diagnosis and the proximity to critical normal organs, which limit the achievable dose of radiation (reviewed in ref. 9). Therefore, radiation has been combined with radiosensitizing chemotherapeutics. However, this combination has yielded only a modest survival benefit and at the cost of significant toxicity in many patients. The epidermal growth factor receptor (EGFR) is a member of the erBB multigene family. Receptor activation is associated with phosphorylation of the intracellular tyrosine kinase domain and recruitment of signaling molecules that initiate the diverse signaling cascades that promote biologic responses, including enhanced proliferation, cell survival, as well as radiation resistance (reviewed in refs. 10, 11). As EGFR is expressed in up to approximately 80% of NSCLC, it constitutes a potentially important target in NSCLC therapy (12). Clinical
trials have sought to integrate EGFR-targeted monoclonal antibodies (mAb) or selective tyrosine kinase inhibitors (TKI) into the treatment of NSCLC to achieve radiosensitization, with varying results (13–15). For NSCLC harboring wild-type EGFR, it is increasingly appreciated that these agents should be selected based on the molecular profile of a given tumor rather than administered to all patients (16). However, predictive biomarkers of radiosensitization have been lacking.

The KRAS gene encodes a GTPase involved in relaying signals from the cell membrane to the nucleus. Upon the introduction of point mutations, most commonly at codons 12 and 13, the K-Ras protein becomes constitutively active and acquires oncogenic properties. KRAS mutations are found in approximately 30% of NSCLC (17) and are associated with resistance to EGFR-targeted agents in monotherapy as well as poor prognosis (18–21). In KRAS-mutant cells, critical prosurvival and growth effector pathways are activated by K-Ras and consequently exhibit resistance to inhibition by TKIs such as erlotinib or mAbs such as cetuximab (22, 23). There is also evidence that KRAS mutations confer radioresistance, although this phenomenon is understudied in NSCLC (24–26).

Large-scale screening of annotated cancer cell lines has been successfully used to identify cell line subsets sensitive to single-agent treatments and associated biomarkers (27–30). There is a need to adapt such screening platforms for the study of IR in conjunction with potentially radiosensitizing targeted compounds. We recently reported that the radiosensitizing effects of erlotinib and cetuximab seen in a short-term survival assay as used in screening platforms correlate well with results of the standard clonogenic survival assay (6). The degree of radiosensitization for single doses of IR was relatively small, i.e., 1.02 to 1.17 for short-term survival and 1.15 to 1.46 for clonogenic survival, consistent with data reported by others (6, 31, 32). However, it is thought that a small radiosensitizing effect incurred by a single dose of IR could be amplified when doses are repeated (33), as would be the case during a several-week clinical course of radiation consisting of 30 or more fractions of approximately 2 Gy each.

Here, we have carried out a screen of 40 human NSCLC-derived cell lines using 2 Gy IR together with erlotinib or cetuximab. We establish a surprising positive association between KRAS mutation and radiosensitization, and we have determined that EGFR-dependent chromatin condensation protects KRAS-mutant cancer cells against IR-induced DSB and premature senescence, thus revealing novel therapeutic opportunities to treat KRAS-mutant cancers.

Materials and Methods

Cell lines

Cell lines were selected from a previously published panel located in the Center for Molecular Therapeutics at Massachusetts General Hospital, Boston, MA (30), except for A549 and Calu-6, which were purchased directly from American Type Culture Collection, and DLD1 (KRAS G13D/wt) and DWT7 (del/wt), which were kind gifts from Dr. Bert Vogelstein, Johns Hopkins University, Baltimore, MD (34). The identity of each of the cell lines in the panel had been tested as described previously (6), and additional cell line authentication was performed by Bio-Synthesis, Inc. No cell line was ever treated for mycoplasma and all lines tested mycoplasma free before the experiments (MycopAlert; Lonza). NCI-H1703 cells harboring wild-type KRAS were transfected with a pBABE-Puro vector encoding KRAS G12V or an empty control (kindly provided by Dr. David Barbie, Dana-Farber Cancer Institute, Boston, MA). Stably transfected clones were selected with 2 mg/mL puromycin (Sigma; P9620) and maintained at 1 mg/mL puromycin. Short hairpin RNA KRAS transfection of A549 cells was performed as described previously (35). For three-dimensional culture of tumor spheres, approximately 5,000 cells per well were grown in low-binding 96-well plates (Thermo; 145399) using serum-free medium composed of Dulbecco’s Modified Eagle’s Medium (Sigma-Aldrich), basic Eagle’s Medium (Sigma-Aldrich), and EGF (20 ng/mL each; Sigma-Aldrich), and B27 supplement (Life Technologies).

Xenograft experiments

A549 xenografts were grown and treated as described previously (6).

Treatments

Irradiation as well as treatments with erlotinib and cetuximab was described previously (6). NU7026 (EMD Millipore; 260961), hesperadin (Selleck; S1529), G66976 LC Laboratories; G-6203), and AZD6244 (Selleck; S1008) were dissolved in dimethyl sulfoxide (DMSO) and chloroquine (Sigma; C6628) was dissolved in double distilled water. Drugs were added 1 hour before irradiation and maintained for the duration of each experiment. Chaetocin (Cayman; CAS 28097) and Ro32-0432 (Merck; 557525) were dissolved in DMSO and incubated with cells for 1 hour before collecting for cell lysates. Thymidine (Sigma; T1895) was dissolved in PBS, and used at 2.5 mmol/L concentration 16 hours before and 8 hours after releasing cells in normal cell culture medium.

Cell proliferation and survival assays

Clonogenic survival assays were performed as previously published (36). Determination of cell numbers 72 hours after irradiation with 2 Gy was performed by manual counting or by using a fluorescent nucleic acid stain Syto60 as described (30). For analyzing tumor spheres, the CellTiter-Glo (CTG) luminescence assay was used (Promega). CTG reagents were applied to lysed spheres 3 days after irradiation following the manufacturer’s protocol. Signals were read by the Multi-Label reader, 2140 Envision (Perkin Elmer).

RNA interference

For depletion of protein kinase C α (PKCα), exponentially growing cells were transfected with validated siPKCα or a scrambled control siRNA (Ambion) using the X-tremeGENE transfection Kit (Roche). Western blotting and subsequent experiments were performed 48 hours after transfection.
Senescence staining
Senescence-associated β-galactosidase staining was performed using a commercial kit (Cell Signaling Technology; #9860) as described previously (6).

Single-cell gel electrophoresis (comet) assay
DSBs were measured at 15 minutes after 2 Gy irradiation by neutral single-cell gel electrophoresis as described previously (37).

Flow cytometry
Cell-cycle distribution analysis and quantification of γ-H2AX signal were performed using flow cytometry as described previously (6).

Immunofluorescence microscopy
Staining and visualization of γ-H2AX and 53BP1 foci were performed as described (38). For visualization of EGFR and trimethyl (Lys9)-phospho (Ser10)-Histone H3 (EMD Millipore; 05-809) expression in vitro and in vivo, the same protocol was used. Following fixing and permeabilization, cells were incubated with γ-H2AX and 53BP1 (Abcam; ab22551 and ab21083, respectively), EGFR (Santa Cruz Biotechnology; sc-03) followed by incubation with an Alexa-488-conjugated secondary antibody (A11029; Invitrogen). All slides were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and examined by fluorescence microscopy (Olympus BX51).

Western blotting
Proteins in whole-cell lysates from exponentially growing cell cultures were detected using standard methods. Specific antibodies against p-EGFR (Tyr992; Invitrogen; 40-8250), trimethylated histone H3 lysine 9 (H3K9me3), dimethylated histone H3 lysine 9 (H3K9me2), monomethylated histone H3 lysine 9 (H3K9me), acetylated histone H3 lysine 9 (H3K9ac), phosphorylation of histone H3 serine 10 (H3S10ph), total histone H3, total-PKCα, and p21 (Abcam; ab88988, ab1220, ab9045, ab12179, ab47297, ab1791, ab23276, and ab18209, respectively), trimethyl (Lys9)-phospho (Ser10)-Histone H3 (EMD Millipore; 05-809), phospho-PKC-pan and Bcl-2 (Cell Signaling Technology; #9371 and #2876), LC3 (MBL; M186-3), (EMD Millipore; 05-809), phospho-PKC-pan (pan) and Bcl-2 (Cell Signaling Technology; #9371 and #2876), LC3 (MBL; M186-3), caspase-3 (Calbiochem; #235412) and β-actin (Sigma-Aldrich; A5411), and horseradish peroxidase–conjugated secondary antibody (Santa Cruz Biotechnology) were used. Protein bands were visualized with enhanced chemiluminescence (Invitrogen) followed by autoradiography.

Transmission electron microscopy
Cells were treated with erlotinib for 1 hour and fixed using standard methods, and transmission electron microscopy was carried out at the MGH Microscopy Core, Program in Membrane Biology. (http://www.partners.org/researchcores/microscopy/microscopy_brown_MGH.asp)

Results
Radiosensitization of cancer cells by EGFR-directed agents is dependent on KRAS mutation
To identify a subset of NSCLC that can be radiosensitized by targeting EGFR, we used a panel of 40 annotated NSCLC cell lines resistant to erlotinib alone (30). Cells were subjected to a clinically relevant dose of IR, 2 Gy, with or without erlotinib or cetuximab treatment (Supplementary Fig. S1A). The number of persisting cells was determined at 72 hours after IR, as described (6). The extent of drug-induced radiosensitization relative to the effect of IR alone was expressed as SRF2Gy (short-term radiosensitization factor at 2 Gy; Supplementary Fig. S1A).

Erlotinib and cetuximab caused radiosensitization with mean SRF2Gy values of 1.10 and 1.13 in 40% and 48% of NSCLC cell lines, respectively (Fig. 1A; Supplementary Fig. S1B). Strikingly, among the top 11 cell lines displaying the greatest radiosensitization by both EGFR-directed agents, eight harbored a mutation in KRAS, even though all cell lines are resistant to erlotinib alone (fraction of cells, >0.5; Supplementary Fig. S1C). When correlating KRAS status with SRF2Gy for all cell lines in the panel, we found a statistically significant increase in mean SRF2Gy in mutant compared with wild-type cell lines for either drug (P < 0.01; Fig. 1B). Genetic manipulation of KRAS in isogenic NSCLC pairs and a colorectal cancer pair (using mutant KRAS overexpression, knockdown, or allelic deletion (Supplementary Fig. S1D; 35) produced similar results (Fig. 1C). Mutant KRAS-specific radiosensitization was even enhanced when drug incubation time before irradiation was extended (Supplementary Fig. S1E). As a confirmation of the 72-hour assay, erlotinib radiosensitized KRAS-mutant but not wild-type NSCLC cells in a clonogenic survival assay (P < 0.01; Fig. 1D), which correlates with EGFR TKI-mediated radiosensitization of KRAS-mutant A549 xenografts (39). Similarly, radiosensitization was seen in KRAS-mutant but not wild-type tumor spheres (Supplementary Fig. S1F).

EGFR suppresses the induction of DSB by promoting chromatin condensation in KRAS-mutant cells
To elucidate the mechanisms of radiosensitization by targeting EGFR, we first examined the induction and removal of DSB using the established DSB marker γ-H2AX in irradiated KRAS-mutant A549 cells similar to previous studies (Fig. 2A; Supplementary Fig. S2A; refs. 5, 40). At 30 minutes after irradiation with 1 Gy, EGFR inhibition by cetuximab or erlotinib increased the percentage of cells with >20 foci per nucleus by 1.2- to 1.6-fold (P = 0.02). This change correlated with an increase in the median number of IR-induced γ-H2AX foci per Gy per nucleus by 1.2- to 1.3-fold within 15 to 30 minutes of irradiation. The EGFR-dependent difference in γ-H2AX induction was confirmed by another DSB marker, 53BP1, and single-cell gel electrophoresis (Fig. 2B). Importantly, the difference in DSB numbers at 30 minutes was not due to a previously proposed effect of EGFR on non-homologous end-joining (NHEJ; 41), as (i) pharmacologic inhibition of DNA-PKcs did not abrogate the difference (Fig. 2C), (ii) EGFR inhibition did not affect nuclear translocation previously hypothesized to represent an EGFR function in NHEJ (Fig. 2D; Supplementary Fig. S2B; 40), and (iii) EGFR activity did not affect NHEJ frequencies in a GFP-based I-sceI reporter assay (Supplementary Fig. S2C). In addition, EGFR...
inhibition was required before irradiation but not immediately afterwards (Fig. 2E). A remarkably consistent and mutant KRAS-dependent increase in DSB upon EGFR inhibition was seen in several nonisogenic and isogenic comparisons, i.e., 1.2- to 1.5-fold difference (Fig. 2F).

We hypothesized that KRAS-mutant cells may harbor more condensed chromatin than wild-type cells, making them less susceptible to accumulate damage to their DNA (42). We, therefore, characterized the impact of erlotinib on chromatin structure using transmission electron microscopy in KRAS-mutant cells (Fig. 3A). EGFR inhibition for 1 hour reduced the fraction of cells with a high content of dense chromatin by 25%. Trimethylation of lysine 9 on histone H3 (H3K9me3) is a general marker of dense chromatin, found in heterochromatin as well as mitotic chromatin (43). Consistent with the electron microscopy findings, EGFR inhibition led to a decrease in the H3K9me3 signal both in vitro and in a xenograft model (Fig. 3B–D). To demonstrate the dependence of DSB induction on chromatin condensation, we pharmacologically inhibited histone methyltransferases (Fig. 3E). The resulting repression of H3K9me3 completely abrogated the effect of EGFR inhibition on DSB induction. Together, these data suggest that EGFR-mediated chromatin condensation protects mutant KRAS cells against IR-induced DSB.

EGFR and PKCα are coregulators of Aurora B kinase-mediated chromatin condensation in interphase

To identify the mechanism by which EGFR may modulate H3K9me3, we first determined the enzymatic activities of histone methyltransferases specifically targeting H3K9 in A549 cells, but there was no effect of EGFR on promoting methyltransferase activity (Supplementary Fig. S3A). We next asked whether EGFR suppresses H3K9 demethylase activities. Although EGFR inhibition did not cause an increase in dimethylation or monomethylation of H3K9, we observed an unexpectedly reduced phosphorylation on H3 serine 10 (p-H3S10; Fig. 4A). Phosphorylation at H3S10 promotes mitotic condensation through H3K9me3 and is generally thought to be a marker of the G2 and M phases of the cell cycle (43, 44). Erlotinib treatment reduced the percentage of cells with colocalized p-H3S10 and H3K9me3 from 18.5% to 8.7% (Fig. 4B), and there was a pronounced signal reduction in vivo as well (Supplementary Fig. S3B). Interestingly, the reduced costaining signal was almost exclusively due to a decrease in the interphase-type, punctuate staining pattern that has been previously described in G2-phase cells (Fig. 4B; ref. 43). To determine whether the observed reduction was indeed specific for G2-phase cells or could be detected in other phases of the cell cycle as well, we performed a double thymidine block, which essentially eliminated G2–M-phase cells (Fig. 4C). Under these conditions, the percentage of cells displaying p-H3S10 and H3K9me3 was reduced by 50% (Fig. 4D).

Figure 1. Lung cancer cell line screening identifies a positive correlation between KRAS mutation and radiosensitization by EGFR-directed agents.
conditions, we continued to observe cells with a colocalized H3K9me3 and p-H3S10 signal that was sensitive to erlotinib. Aurora B kinase phosphorylates H3S10 to promote mitotic condensation, but the existence of this modification outside of the G2–M-phase is unknown. Pharmacologic inhibition of Aurora B kinase abrogated not only mitotic colocalization of p-H3S10 and H3K9me3 but also the interphase-type staining pattern (Supplementary Fig. S3C). Furthermore, consistent with the data in Fig. 4C, Aurora B kinase inhibition increased the amount of IR-induced DSB in G1 phase cells, with no additional effect of EGFR inhibition on the DSB signal (Fig. 4D). DSB induction resulting from Aurora B kinase inhibition was more pronounced in KRAS-mutant than in wild-type cells, with the latter effect likely representing a modification in mitotic cells (Fig. 4E).

We conclude that Aurora B kinase and EGFR promote a mitosis-like chromatin condensation mechanism in a fraction of interphase KRAS-mutant cells, thereby suppressing DSB induction.

Lastly, we wanted to identify the EGFR signaling pathway that promotes the inhibition of DSB induction. We, therefore, screened KRAS-mutant A549 cells with a panel of pharmacologic inhibitors directed at the key pathways known to function downstream of EGFR (Supplementary Fig. S3D). Only inhibition of protein kinase C alpha (PKCα) was found to affect DSB induction, and the effect was epistatic with EGFR inhibition (Fig. 4F). The increase in DSB induction by a PKCα inhibitor was abolished in cells in which PKCα was depleted by RNA interference (Fig. 4G). As predicted, PKCα inhibition reduced H3K9me3 levels to a degree comparable with EGFR inhibition (Fig. 4H; Supplementary Fig. S3E). Analogous to erlotinib, PKCα inhibition repressed the colocalized p-H3S10 and H3K9me3 signal and correspondingly increased DSB induction in NCI-H1703 cells expressing mutant KRAS, but not in isogenic wild-type cells (Fig. 4I; Supplementary Fig S3F).

A senescence-to-apoptosis switch in cells expressing mutant K-Ras

In an isogenic model, KRAS wild-type cells were sensitive to treatment with erlotinib alone (Supplementary Fig. S4A), with the induction of premature cellular senescence accounting for at least some of the effect (Fig. 5A). In contrast, erlotinib-treated KRAS-mutant cells were resistant to senescence induction. However, upon additional irradiation with 2 Gy, the senescence response was reactivated (while IR alone did not cause senescence; Fig. 5A). Consistent with our previous results (6), MEK–ERK inhibition caused a small degree of senescence in KRAS-mutant cells that could not explain all the effect of erlotinib treatment (Fig. 5B; Supplementary Fig. S4B).
reported to exhibit increased autophagy activity and that death, we considered that KRAS-mutant cells have been secrete growth factors. To convert senescence to apoptotic cell desirable effect as senescent tumor cells remain viable and can produced the full senescence phenotype.

As a result, the radiosensitizing effect of combined erlotinib and chloroquine was observed only in the presence of KRAS mutation and was particularly pronounced in KRAS-mutant tumor spheres where the average $SR_{2Gy}$ reached 2.0 (Fig. 5F).

**Discussion**

KRAS is the most commonly mutated oncogene in NSCLC. KRAS-mutant NSCLCs often display a poor prognosis and radioresistance, and have proved refractory to most targeted therapies, including EGFR-directed agents. These cancers thus remain a major clinical challenge. Here, we report that in the context of a DSB-producing treatment, EGFR assumes an important role in promoting the survival of KRAS-mutant cancer cells, thus rendering these cells susceptible to EGFR inhibitors (Fig. 1, Fig. 2). Interestingly, a previously understudied mechanism underlying the radiosensitizing effect of EGFR inhibition involves the modulation of the number of DSB generated by a given dose of IR, which is seen in all analyzed KRAS-mutant cell lines (Fig. 2F). It is generally accepted that the cytotoxic effects of IR result principally from damage to DNA (3). The majority of DNA breaks from X-rays or photon radiation as used in the clinic is caused by indirect action, meaning IR-induced free radicals that diffuse far enough to damage a DNA molecule. It can thus be postulated that regions of decondensed, open chromatin would make the exposed DNA helix more vulnerable to radiation ionizations, whereas condensed regions would be relatively less susceptible to DNA damage induction (42). Consistent with this concept, EGFR inhibition loses its effect on DSB induction in the setting of open chromatin (Fig. 3E).

Unexpectedly, the EGFR-mediated reduction of DSB induction was characterized by colocalized H3K9me3 and p-H3S10 (Fig. 4B), which is an established marker of mitotic chromatin condensation during late $G_2$ and mitosis. Interestingly, we not only found that this chromatin mark is also present in the $G_1$ phase in a subset of cells but also that Aurora B suppresses DSB induction in $G_1$ (Fig. 4C and D). It was previously shown that among the key mitotic regulators, Aurora B is degraded by the anaphase-promoting complex/cyclosome ubiquitin ligase at a relatively late stage and is still detectable in the $G_1$ phase (47). Our results suggest that Aurora B remains active in $G_1$ to maintain condensed chromatin in a fraction of cells, a process that is coregulated by an EGFR–PKCα-dependent pathway, thereby protecting KRAS-mutant cells against DSB through most of the cell cycle (Fig. 4D).

**What might be the purpose of enhanced chromatin condensation in KRAS-mutant NSCLC cells?**

Oncogene-induced...
senescence is a crucial tumor suppressor mechanism controlling unchecked proliferation driven by oncogenic mutations such as KRAS (48). During carcinogenesis, oncogene-driven cells can escape senescence by inactivation of the p53 or p16/BB pathways. However, these genetic events may not codevelop senescence responses that are inducible by DSB (i.e., "stress-induced premature senescence"; ref. 8). It is important to note that although exogenous DSB can be induced by...
IR, endogenous DSBs arise as byproducts of normal intracellular metabolism. The spontaneous rate of endogenous DSBs may be as high as 50 DSBs per cell per cell cycle (49), and conceivably could be even higher in cells with activated oncogenes such as mutant KRAS that drive cells through the cell cycle without sufficient time for damage repair. We observed that neither low doses of IR (2 Gy, causing 40–80 DSB) nor EGFR inhibition alone induced senescence in KRAS-mutant cells, but, unexpectedly, the combination of these treatments did (Fig. 5A). These data support a model in which KRAS-mutant cells use EGFR–PKCα for suppression of a DSB-induced senescence response, which may be important during the process of carcinogenesis, yet represents a therapeutic target.

Although there is currently great interest in exploiting stress-induced premature senescence for anticancer therapy, it is important to realize that senescent cells remain alive and may even escape senescence (7). Interestingly, recent studies have suggested that autophagy may be a potential promoter or a precondition of stress-induced premature senescence (50, 51). At the same time, autophagy may inhibit apoptosis in tumor cells consistent with a cytoprotective role (46). Consistent with these data, we discovered that autophagy inhibition by chloroquine converts the senescence response to a more desirable apoptotic outcome (Fig. 5C–E). Importantly, the combination of EGFR TKI and chloroquine has been already explored in the clinic and shows a favorable toxicity profile (52). Therefore, combining IR with EGFR-directed therapy and an autophagy inhibitor such as chloroquine may warrant clinical investigation in KRAS-mutant cancers. In conclusion, our data challenge the current paradigm of EGFR inhibitor resistance in KRAS-mutant cancers and, by providing insight into the role of EGFR in chromatin modulation and senescence suppression, yield potential novel therapeutic opportunities.

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

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Conception and design: M. Wang, M. Hülskötter, M. Krause, M. Baumann, J. Settleman, H. Willers
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Figure 5. A senescence-to-apoptosis switch in KRAS-mutant cancer cells. A, representative images (~40) showing staining for DAPI and senescence-associated β-galactosidase (SA-β-gal) 3 days following 2 Gy irradiation in DLD1 (KRAS wt/mut) or DWT7 (wt/-) cells. B, percentage of A549 cells staining for SA-β-gal staining 7 days following 2 Gy irradiation ± erlotinib ± the MEK inhibitor AZD6244 (250 nmol/L) or Go6976. C, whole-cell lysates from A549 cells 72 hours after irradiation (8 Gy) ± erlotinib (2 μmol/L) or ± chloroquine (CQ; 25 mmol/L) were subjected to Western blotting with antibodies against proteins as shown. D, representative images for DAPI and SA-β-gal staining obtained in parallel to the data shown in plot D. E, percentage of sub-G₁ cells as determined by FACS in KRAS-mutant DLD-1 and wild-type DWT7 cells for the treatments indicated. F, SRF2G values for DLD-1 and DWT7 tumor spheres treated as indicated. Statistical comparisons by one-sample or unpaired t test. In all plots, bars, mean ± SE based on typically two to three biologic repeats.
EGFR Protects KRAS-Mutant Cells

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