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Report

Intrinsic Resistance to MEK Inhibition in *KRAS* Mutant Lung and Colon Cancer through Transcriptional Induction of *ERBB3*

Chong Sun,[1] Sebastijan Hobor,[2] Andrea Bertotti,[2,3] Davide Zecchin,[2,3] Sidong Huang,[1,4] Francesco Galimi,[2,3] Francesca Cottino,[2] Anirudh Prahallad,[1] Wipawadee Grernrum,[1] Anna Tzani,[1] Andreas Schlicker,[1] Lodewyk F.A. Wessels,[1] Egbert F. Smit,[5] Erik Thunnissen,[6] Pasi Halonen,[1] Cor Lieftink,[1] Roderick L. Beijersbergen,[1] Federica Di Nicolantonio,[3] Alberto Bardelli,[2,3,7,*] Livio Trusolino,[2,3] and Rene Bernards[1,*]

[1]Division of Molecular Carcinogenesis, Cancer Genomics Center Netherlands, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, the Netherlands
[2]Candiolo Cancer Institute - FPO, IRCCS, Strada Provinciale 142 km 3.95, 10060 Candiolo, Torino, Italy
[3]Department of Oncology, University of Torino, Strada Provinciale 142 km 3.95, 10060 Candiolo, Torino, Italy
[4]Department of Biochemistry, The Rosalind and Morris Goodman Cancer Centre, McGill University, Montreal, QC H3G 1Y6, Canada
[5]Department of Pulmonary Diseases, VU University Medical Centre, P.O. Box 7057, 1007 MB Amsterdam, the Netherlands
[6]Department of Pathology, VU University Medical Centre, P.O. Box 7057, 1007 MB Amsterdam, the Netherlands
[7]FIRC Institute of Molecular Oncology (IFOM), 20139 Milano, Italy
*Correspondence: alberto.bardelli@ircc.it (A.B.), r.bernards@nki.nl (R.B.)
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SUMMARY

There are no effective therapies for the ~30% of human malignancies with mutant *RAS* oncogenes. Using a kinome-centered synthetic lethality screen, we find that suppression of the *ERBB3* receptor tyrosine kinase sensitizes *KRAS* mutant lung and colon cancer cells to MEK inhibitors. We show that MEK inhibition results in MYC-dependent transcriptional upregulation of *ERBB3,* which is responsible for intrinsic drug resistance. Drugs targeting both EGFR and ERBB2, each capable of forming heterodimers with ERBB3, can reverse unresponsiveness to MEK inhibition by decreasing inhibitory phosphorylation of the proapoptotic proteins BAD and BIM. Moreover, ERBB3 protein level is a biomarker of response to combinatorial treatment. These data suggest a combination strategy for treating *KRAS* mutant colon and lung cancers and a way to identify the tumors that are most likely to benefit from such combinatorial treatment.

INTRODUCTION

Cancer treatment is gradually changing from an organ-centered to a pathway-centered approach. Cancer cells are often addicted to signals generated by cancer-causing genes. Consequently, targeted cancer drugs that selectively inhibit the products of activated oncogenes can have dramatic effects on cancer cell viability (Weinstein, 2002). This approach has yielded significant clinical results for non-small-cell lung cancer (NSCLC) that have activating mutations in *EGFR* (Lynch et al., 2004) or translocations of the *ALK* kinase (Kwak et al., 2010) and for melanoma patients with *BRAF* mutant tumors (Flaherty et al., 2010). Some 20%–30% of all human malignancies have oncogenic mutations in a *RAS* gene family member (Bos, 1989), but pharmacological inhibition of RAS proteins in the clinic remains challenging. An alternative approach to targeting mutant RAS involves using small molecule inhibitors targeting downstream RAS effectors: the RAF-MEK-ERK kinases. However, to date, the results of MEK inhibition in cancer have been modest, both in the clinic and in patient-derived xenograft models (Adjei et al., 2008; Jänne et al., 2013; Migliardi et al., 2012). Such a lack of response to inhibition of a pathway that is activated in cancer may result from feedback activation of the inhibited pathway or a secondary pathway that supports cancer cell viability in the presence of the inhibitory drug (reviewed in Bernards, 2012).

Therefore, we set out to search for kinases whose inhibition is synthetic lethal with MEK inhibition in both *KRAS* mutant NSCLC, a form of cancer in which this gene is activated with a frequency of around 30% (Bos, 1989), and in *KRAS* mutant colon cancer, where *KRAS* mutational activation occurs in more than 40% of cases (Pylayeva-Gupta et al., 2011). Using a kinome-centered synthetic lethality screen in a *KRAS* mutant NSCLC cell line, we now identify kinases whose inhibition is synthetic lethal when combined with MEK inhibition.

RESULTS

KRAS Mutant Cancer Cell Lines Are Unresponsive to MEK Inhibitors

To study how *KRAS* mutant cancer cells respond in vitro to MEK inhibition, we determined the efficacy of the MEK inhibitor selumetinib (AZD6244) in four NSCLC and four colon cancer cell lines using a long-term proliferation assay. Figure 1A shows that all but one colon cancer cell line were relatively insensitive to selumetinib. Consistent with this, the vast majority of the *KRAS*

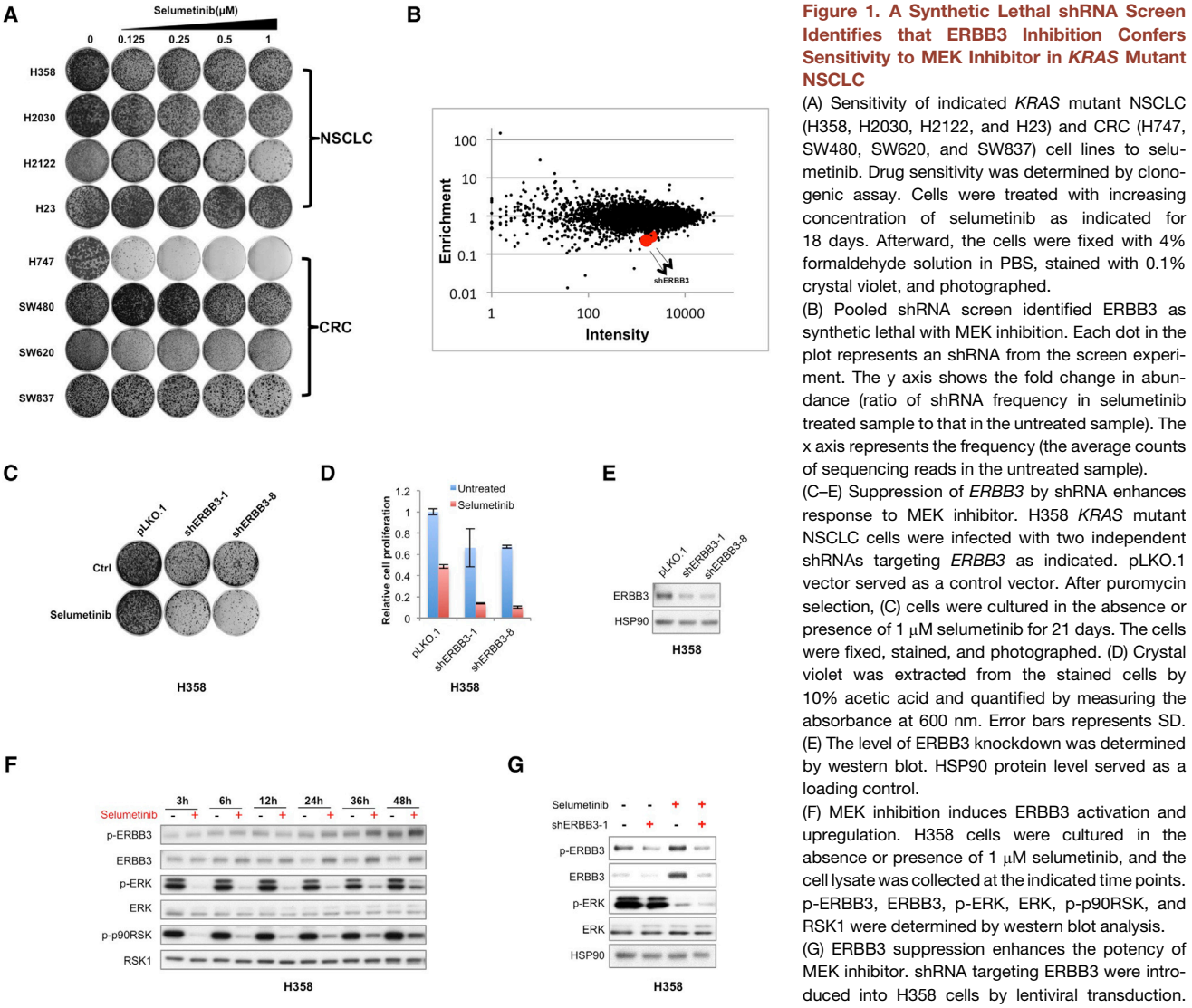

Figure 1. A Synthetic Lethal shRNA Screen Identifies that ERBB3 Inhibition Confers Sensitivity to MEK Inhibitor in *KRAS* Mutant NSCLC

(A) Sensitivity of indicated *KRAS* mutant NSCLC (H358, H2030, H2122, and H23) and CRC (H747, SW480, SW620, and SW837) cell lines to selumetinib. Drug sensitivity was determined by clonogenic assay. Cells were treated with increasing concentration of selumetinib as indicated for 18 days. Afterward, the cells were fixed with 4% formaldehyde solution in PBS, stained with 0.1% crystal violet, and photographed.

(B) Pooled shRNA screen identified ERBB3 as synthetic lethal with MEK inhibition. Each dot in the plot represents an shRNA from the screen experiment. The y axis shows the fold change in abundance (ratio of shRNA frequency in selumetinib treated sample to that in the untreated sample). The x axis represents the frequency (the average counts of sequencing reads in the untreated sample).

(C–E) Suppression of *ERBB3* by shRNA enhances response to MEK inhibitor. H358 *KRAS* mutant NSCLC cells were infected with two independent shRNAs targeting *ERBB3* as indicated. pLKO.1 vector served as a control vector. After puromycin selection, (C) cells were cultured in the absence or presence of 1 μM selumetinib for 21 days. The cells were fixed, stained, and photographed. (D) Crystal violet was extracted from the stained cells by 10% acetic acid and quantified by measuring the absorbance at 600 nm. Error bars represents SD. (E) The level of ERBB3 knockdown was determined by western blot. HSP90 protein level served as a loading control.

(F) MEK inhibition induces ERBB3 activation and upregulation. H358 cells were cultured in the absence or presence of 1 μM selumetinib, and the cell lysate was collected at the indicated time points. p-ERBB3, ERBB3, p-ERK, ERK, p-p90RSK, and RSK1 were determined by western blot analysis.

(G) ERBB3 suppression enhances the potency of MEK inhibitor. shRNA targeting ERBB3 were introduced into H358 cells by lentiviral transduction. Cells were cultured in medium either with or without 1 μM selumetinib for 24 hr before the harvest for western blot analysis.

mutant cancer cell lines present in the Sanger and CCLE cell line encyclopedias (Barretina et al., 2012; Garnett et al., 2012) have an IC_{50} for selumetinib of over 1 μM (Figures S1A and S1B). Together, these cell line data recapitulate the animal studies and the early-phase clinical trial data that show only a modest activity of MEK inhibition in *KRAS* mutant tumors (Adjei et al., 2008; Jänne et al., 2013; Migliardi et al., 2012).

A Synthetic Lethal Screen with MEK Inhibitor

We have recently described the use of a kinome-centered synthetic lethal screening approach, which enables the identification of kinases whose inhibition is strongly synergistic with a cancer drug of interest (Prahallad et al., 2012). In brief, in such a genetic screen a collection of 3,530 short hairpin RNA (shRNA) vectors that collectively target all 518 human kinases for suppression through RNA interference is introduced into cancer cells through lentiviral infection. Each of these knockdown vectors has a unique DNA-based molecular bar code identifier, which allows quantification of the relative abundance of each of the shRNA vectors in the presence and absence of drug (Prahallad et al., 2012). To find kinases whose suppression synergizes with selumetinib in *KRAS* mutant NSCLC, we infected selumetinib-resistant H358 cells with the kinome shRNA library and cultured cells both in the presence and absence of selumetinib. After 21 days, genomic DNA was isolated from both cells of the treated and untreated populations, and the bar codes contained in the shRNA cassettes were recovered by PCR, and their abundance was determined by deep sequencing. For hit selection, only shRNAs were included for which total mean read frequencies were over 1,000. To minimize the chance in identifying off-target effects, hits were selected based on the presence of at least two individual shRNAs targeting the same gene in the top

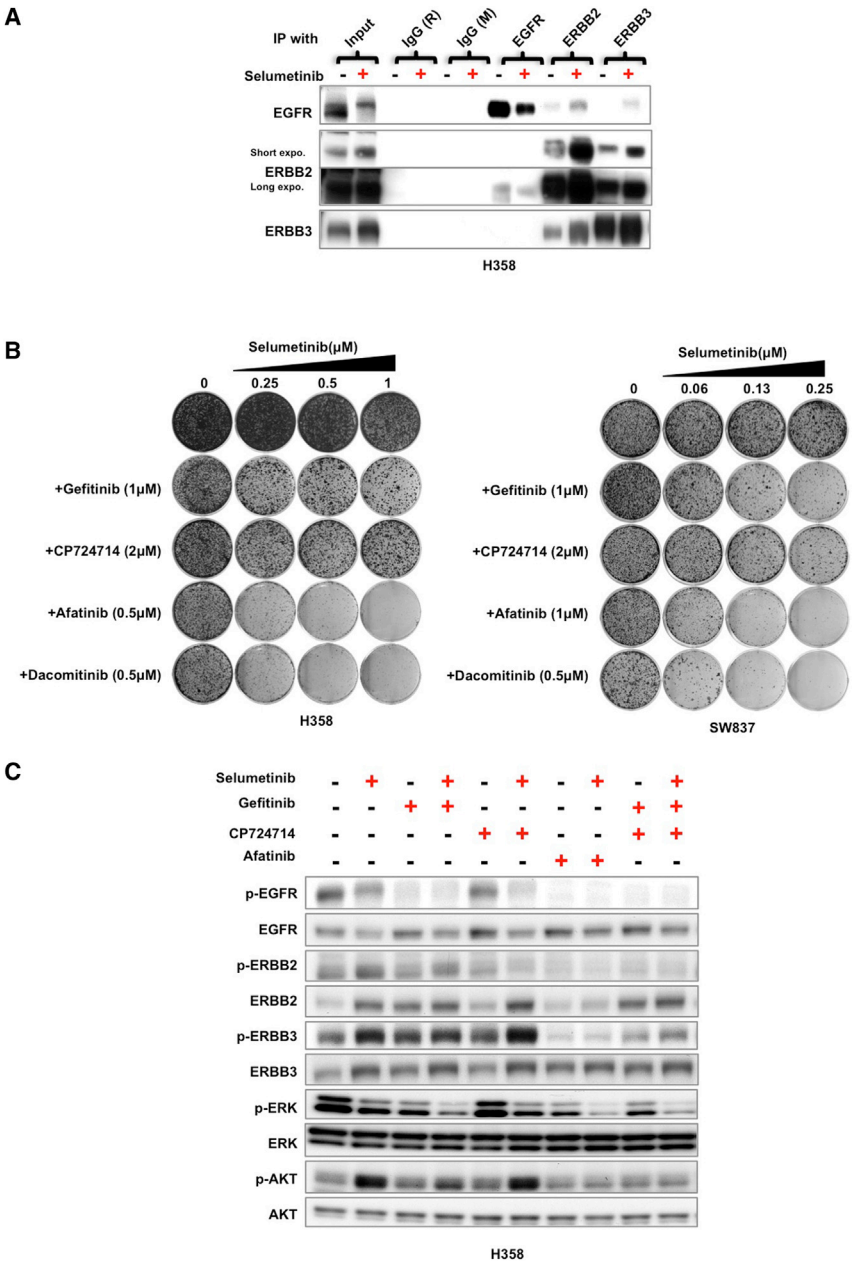

Figure 2. Simultaneous Suppression of EGFR and ERBB2 Sensitizes *KRAS* Mutant Cancer Cells to MEK Inhibitor

(A) MEK inhibitor treatment increases ERBB3-EGFR and ERBB3-ERBB2 heterodimers. H358 cells were cultured with or without 1 μM selumetinib for 36 hr. Immunoprecipitation was performed using EGFR-, ERBB2-, or ERBB3-specific antibody. Rabbit and mouse immunoglobulin G (IgG) served as control antibodies. The precipitates were immunoblotted for EGFR, ERBB2, and ERBB3. Two exposures of the ERBB2 western blot are shown.

(B) Dual EGFR/ERBB2 inhibitors potentiate MEK inhibitor. H358 NSCLC cell and SW837 CRC cell were cultured in increasing concentration of MEK inhibitor selumetinib alone, EGFR inhibitor gefitinib alone, ERBB2 inhibitor CP724714 alone, EGFR/ERBB2 dual inhibitor afatinib alone, or their combinations as indicated. Cells were harvested, fixed, and stained after 21 days.

(C) Effects of pharmacological inhibition of EGFR, ERBB2, MEK, and their combinations. Cells were treated with selumetinib, gefitinib, CP724714, afatinib, and their combinations as indicated for 36 hr. Responses of cells were examined by western blot analysis with the indicated antibodies.

list. Two independent shRNA vectors targeting the EGFR-related kinase *ERBB3* were among the top depleted shRNA vectors on this list (Figure 1B; Table S1). To validate this finding, we infected H358 cells with two *ERBB3* shRNA vectors (both of which reduced *ERBB3* levels [Figures 1C–1E]) and cultured these cells with or without selumetinib for 21 days. Inhibition of *ERBB3* only had minor effects on proliferation of H358 cells, but suppression of *ERBB3* in combination with selumetinib caused a marked inhibition of proliferation in H358 cells (Figures 1C and 1D). Consistently, we observed that MEK inhibitor treatment lead to ERBB3 activation and upregulation, which coincided with ERK reactivation (Figure 1F); ERBB3 suppression enhanced MEK inhibitor efficacy by further reducing ERK activity (Figure 1G). Similar results were obtained in *KRAS* mutant SW480 and SW837 colon cancer cells and H2030 and H2122 NSCLC cells (Figures S1C–S1F).

Dual EGFR/ERBB2 Inhibitors Synergize with MEK Inhibitors

ERBB3 is the only kinase-defective member of the *ERBB* RTK gene family that consists of four members: ERBB1–4. ERBB3 can form heterodimeric active kinase complexes with other members of the ERBB family (Sithanandam and Anderson, 2008). We found that selumetinib treatment of H358 cells caused a marked increase in both ERBB3 and ERBB2 protein (Figures 2C and 3A). Similar results were obtained in SW837 colon cancer cells and H2030 NSCLC, suggesting this is a common response to MEK inhibition in both *KRAS* mutant lung and colon cancer (Figures 3A and S2B). This resulted in an increase in EGFR-ERBB3 and ERBB2-ERBB3 heterodimeric complexes, as judged by coimmunoprecipitation (Figure 2A). To ask which of these two heterodimeric complexes could be responsible for the poor response to selumetinib, we treated both H358 cells and SW837 cells with a combination of selumetinib and gefitinib (an EGFR inhibitor) or the combination of selumetinib and CP724714 (an ERBB2 inhibitor). Neither of these two combinations showed strong synergy in long-term proliferation assays, but the dual EGFR-ERBB2 inhibitors afatinib and dacomitinib each showed strong synergy with MEK inhibition, both in the H358 cells and in SW837 cells (Figure 2B). Similar results

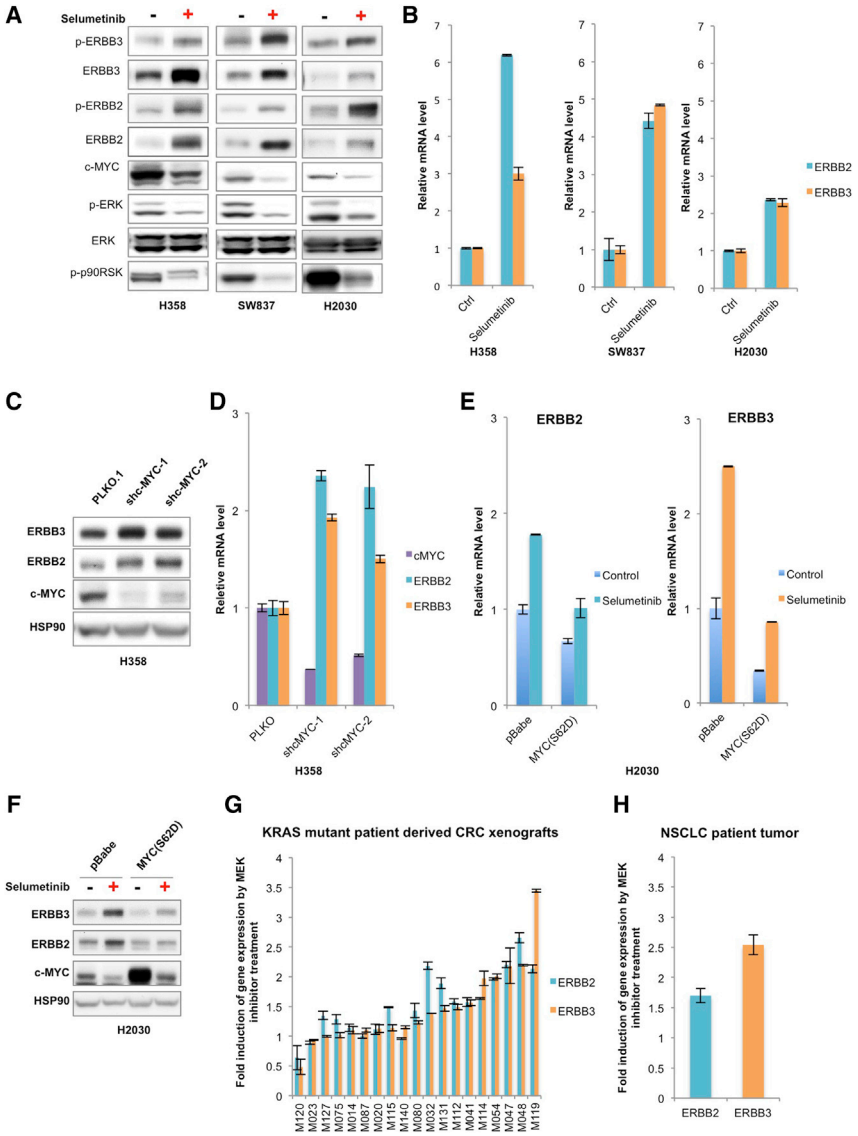

Figure 3. MEK Inhibition Relieves a MYC-Dependent Transcriptional Repression of ERBB3

(A and B) MEK inhibition causes MYC degradation and ERBB2 and ERBB3 upregulation. Cells were treated with 1 μM selumetinib for 24–48 hr before the cell lysate was collected for (A) western blot analysis with the indicated antibodies or (B) qRT-PCR analysis for expression of *ERBB2* and *ERBB3*.

(C and D) MYC suppression leads to ERBB2 and ERBB3 upregulation. Cells were infected with two independent shRNAs targeting MYC. pLKO.1 vector served as control. After puromycin selection, cells were subjected to (C) western blot or (D) qRT-PCR analysis to measure expression of *MYC*, *ERBB2*, and *ERBB3*.

(E and F) Ectopic expression of MYC(S62) blocks MEK inhibitor induced ERBB2 and ERBB3 upregulation. MYC(S62D) was introduced to H2030 NSCLC cells by retroviral transduction. pBabe empty vector served as control. Cells were treated with 1 μM selumetinib for 36 hr before the harvest for (E) qRT-PCR and (F) western blot analysis for ERBB2 and ERBB3 expression.

(G) Induction of *ERBB2* and *ERBB3* in *KRAS* mutant CRC patient-derived xenografts (PDX) following in vivo treatment with selumetinib. The 19 cases were derived from different patients, either untreated or treated with selumetinib (25 mg/kg QD) for 3 or 6 weeks. Mice were systematically sacrificed no later than 4 hr after the last drug administration. Tumor samples were fresh frozen and subjected to RNA isolation and human-specific TaqMan probe-based gene expression analysis afterward.

(H) Induction of *ERBB2* and *ERBB3* by MEK inhibitor treatment in paired biopsies (before and during trametinib treatment) from a *KRAS* mutant NSCLC patient. Tumor biopsy specimens were formalin fixed, paraffin embedded. After RNA isolation, *ERBB2* and *ERBB3* expression levels were determined by TaqMan probe-based gene expression analysis. Error bars represent mean ± SD.

were seen in three additional *KRAS* mutant cells lines: SW620 (colon), H2030 (lung), and H2122 (lung, Figure S2A). Moreover, a second MEK inhibitor (GSK1120212, trametinib) also showed strong synergy with afatinib in four different *KRAS* mutant colon and lung cancer cell lines (Figure S4A). We conclude that MEK inhibition leads to the formation of kinase-active EGFR-ERBB3 and ERBB2-ERBB3 heterodimeric complexes and that both need to be inhibited to enable colon cancer and lung cancer cells to respond to MEK inhibition. This conclusion is further supported by the notion that only the combination of shRNA vectors against both *EGFR* and *ERBB2* synergize with selumetinib, but not either shRNA vector alone (Figures S2E and S2F).

MYC Inhibition Relieves Transcriptional Repression of *ERBB3*

Selumetinib caused an increase in both total ERBB3 and active phospho-ERBB3 (p-ERBB3) in both H358 and in SW837 cells, and similar effects were seen for ERBB2 (Figures 2C and 3A). MEK-ERK signaling is known to enhance stability of MYC through phosphorylation of the serine 62 residue (Sears et al., 1999, 2000). Moreover, MYC has been shown to be a negative regulator of *ERBB2* transcription (Suen and Hung, 1991). Induction of ERBB3 was first seen around 12–24 hr postselumetinib exposure, indicating that a transcriptional response may be involved in the activation of this receptor (Figures 1F and S1G). Indeed, inhibition of MEK by selumetinib caused a decrease in MYC protein in both NSCLC and colon cancer cells, and this was accompanied by an increase in both *ERBB2* and *ERBB3* mRNA expression in multiple *KRAS* mutant cell lines of lung and colon (Figures 3A, 3B, S3A, and S3B). In addition, knockdown of *MYC* by two independent shRNAs caused a reduction in MYC protein and an increase in both *ERBB2* and *ERBB3* mRNA and protein (Figures 3C, 3D, and S3C).

Consistent with a role for MYC SER62 phosphorylation in induction of *ERBB2* and *ERBB3*, we found that expression of the phosphomimetic mutant MYC (SER62D) (Wang et al., 2010) effectively blocked induction of both ERBB2 and ERBB3 by selumetinib (Figures 3E and 3F). The induction of ERBB2 and ERBB3 is most likely primarily at the level of transcription, as ectopic expression of V5-tagged versions of these proteins were not affected in their abundance by MEK inhibition (Figure S3D). Moreover, we could exclude a role for CtBP1 and CtBP2 as well as FOXD3 in regulation of the ERBB proteins in response to MEK inhibition (Figures S3F and S3G), because these genes have been implicated in ERBB3 regulation in other cancer types (Abel et al., 2013; Montero-Conde et al., 2013). Induction of *ERBB2* and *ERBB3* was also seen in half of 19 independent patient-derived xenografts from *KRAS* mutant colorectal cancers in response to MEK inhibition in vivo (Figures 3G and S3E) (Migliardi et al., 2012). Finally, we were able to obtain a paired biopsy from a patient having a *KRAS* mutated adenocarcinoma of the lung before and after 1 week of treatment with the MEK inhibitor trametinib in the context of a randomized phase II clinical trial. Here, we observed induction of both *ERBB2* and *ERBB3* by MEK inhibitor treatment, suggesting that this transcriptional RTK activation is potentially also limiting responses to MEK inhibition in the clinic (Figure 3H).

Synergistic Inhibition of ERK Causes Apoptosis through Activation of BAD and BIM

To address the mechanism by which selumetinib and afatinib synergize to reduce viability of *KRAS* mutant lung and colon cancer cells, we assayed induction of apoptosis over a 4 day period in real time in the presence of selumetinib, afatinib, or the combination of both drugs. Both the H358 and SW837 cells displayed only modest evidence of apoptosis following drug monotherapy, but strongly synergistic induction of apoptosis when selumetinib and afatinib were combined (Figures 4A and 4B). Consistently, both drugs were also highly synergistic in induction of cleaved PARP, a hallmark of apoptotic cells (Figures 4C and 4D).

The RAF-MEK-ERK signaling cascade inhibits apoptosis in part through induction of proapoptotic factors BAD and BIM (Zha et al., 1996) (Corcoran et al., 2013). MEK-ERK inhibition induces BIM and decreases inhibitory phosphorylation of the BAD, which can heterodimerize with BCL-XL and BCL-2, neutralizing their protective effect and promoting cell death. Only the nonphosphorylated BAD forms heterodimers that promote cell death (Zha et al., 1996). BAD can be phosphorylated both by the MEK-ERK and the PI3K-AKT signaling routes on SER112 and SER136, respectively (Bonni et al., 1999; Datta et al., 1997; Scheid et al., 1999). Consistent with the finding that afatinib and selumetinib synergize to inhibit ERK signaling (Figures 2C, 4C, and 4D), we also observed a clear synergistic inhibition of p-BAD SER112 by these two drugs. Moreover, adding afatinib suppressed AKT signaling and also BAD SER136 phosphorylation (Figures 4C and 4D). In addition, we see induction of BIM by MEK inhibition and decreased p-BIM SER69 upon ERK inhibition (Figures 4C and 4D).

In Vivo Validation of the Combination Therapy

We tested the effects of the drug combinations discussed above on *KRAS* mutant NSCLC and CRC cells in vivo. We used the MEK inhibitor trametinib, which showed the same synergy with afatinib in vitro as selumetinib (Figure S4A). As can be seen in Figures 4E and 4F, we observed a modest inhibition of tumor growth when the MEK inhibitor and the dual EGFR-ERBB2 inhibitor afatinib were used alone and a complete inhibition of tumor growth over prolonged time when the two drugs were given together. The drug combination was well tolerated over the 4 week treatment period (Figure S4D). Moreover, in two independent patient-derived xenograft models of *KRAS* mutant CRC, we also observed that both drugs combined were more effective in the inhibition of tumor growth than either drug alone (Figures S4F and S4G). In these models, we also observed increases in ERBB2 and ERBB3 mRNA and protein upon MEK inhibition (Figures S4B, S4C, and S4H–S4K).

Biomarker of Response to the Combination Therapy

To ask whether *KRAS* mutant CRCs and NSCLCs are heterogeneous in their responses to combined MEK and EGFR+ERBB2 inhibition, we determined the degree to which combination of MEK inhibitor and afatinib were synergistic in inhibition of proliferation of 21 CRC and NSCLC cell lines. We calculated the synergy scores for the combination of MEK inhibition and afatinib for all cell lines (Table S2). The synergy score was low in cells having low basal levels of ERBB3 protein and high for cells having high basal ERBB3 expression (Figures 4G, 4H, and S4E). Altogether, our data suggest a combination therapy for the treatment of *KRAS* mutant NSCLC and colon cancers. Moreover, tumors having high basal ERBB3 expression are most likely to benefit from this combination.

DISCUSSION

We have used a kinome-centered synthetic lethality screen to identify potential kinases whose inhibition is synergistic with MEK inhibition in the treatment of *KRAS* mutant NSCLC and colon cancers. Our data identify the Receptor Tyrosine Kinase family member *ERBB3* as a prominent "hit" in this screen with the MEK inhibitor selumetinib. ERBB3 is not an active kinase itself but forms active heterodimeric complexes with one of the three other gene family members: *ERBB1* (*EGFR*), *ERBB2* (*HER2*), and *ERBB4* (which is primarily expressed in the brain). Our data indicate that MEK inhibition in *KRAS* mutant cancer cells of lung and colon leads to degradation of MYC, consistent with the established role for MEK-ERK signaling in stabilizing MYC through phosphorylation of MYC serine 62 (Sears et al., 1999, 2000). MYC is also known to act as a transcriptional repressor of *ERBB2* (Suen and Hung, 1991). We find that suppression of *MYC* not only activates *ERBB2*, but also *ERBB3*, indicating that MYC also acts as a repressor of *ERBB3*. Consequently MEK inhibition causes a transcriptional upregulation of both *ERBB2* and *ERBB3* and the formation of kinase-active ERBB1-ERBB3 and ERBB2-ERBB3 heterodimeric complexes that activate downstream PI3K-AKT and MEK-ERK signaling. We found that inhibition of EGFR or ERBB2 alone with small molecules did not synergize with MEK inhibition, whereas dual

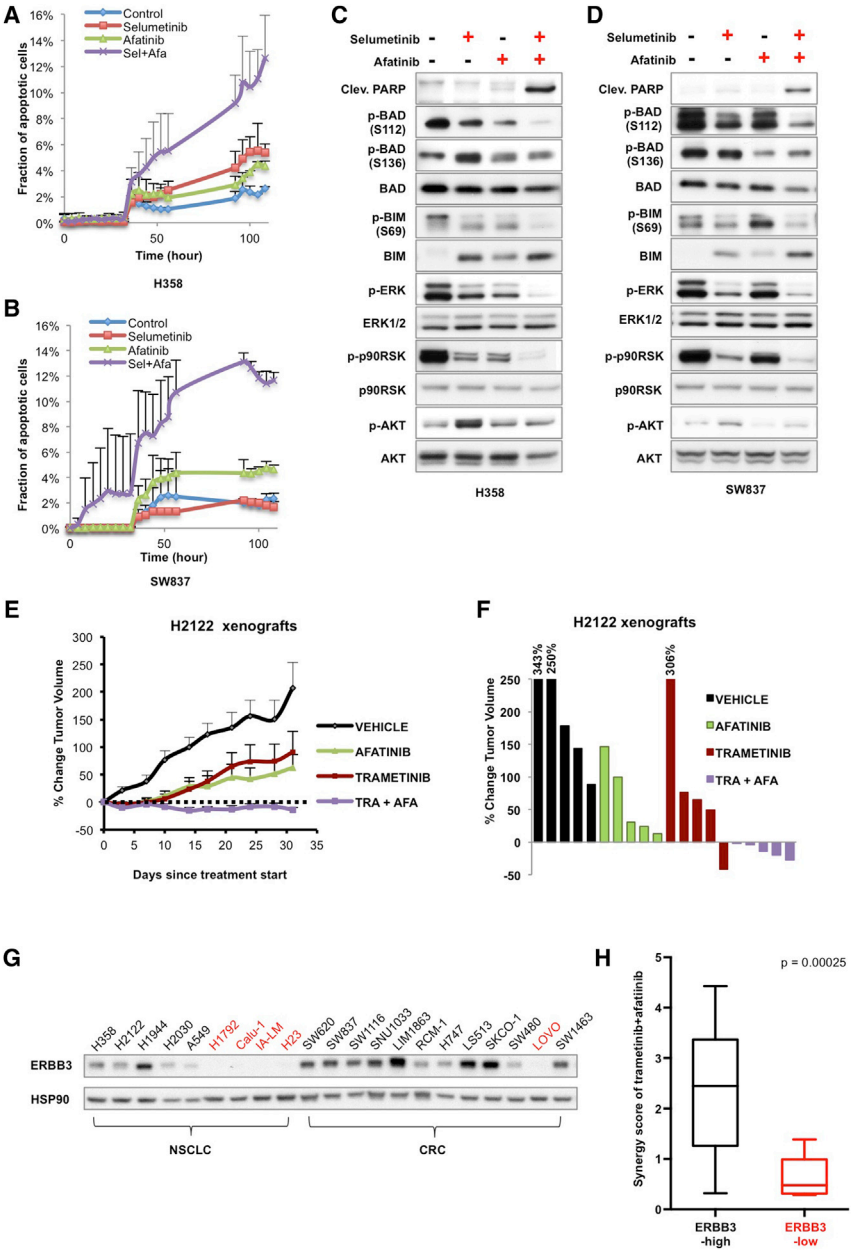

Figure 4. EGFR/ERBB2 Inhibitor and MEK Inhibitor Induce Apoptosis through Activation of BAD and BIM

(A and B) H358 or SW837 cells were cultured with normal medium or medium containing 1 μM selumetinib, 1 μM afatinib or their combination. Apoptotic cells were determined by CellPlayer kinetic caspase-3/7 apoptosis assay according to the user manual.

(C and D) Cells were treated with 1 μM afatinib, 1 μM selumetinib, or the combination for 48 hr. Western blot analysis was performed with indicated antibodies to determine the biochemical response.

(E) H2122 *KRAS* mutant NSCLC cells were injected subcutaneously in nude mice. Once tumors were established, animals (five per group) were treated with vehicle, afatinib (12.5 mg/kg daily), trametinib (1 mg/kg daily), or both drugs in combination (TRA+AFA). The mean percentage change in tumor volume relative to initial tumor volume is shown. Error bars represent mean ±SEM.

(F) Waterfall plot showing the percentage change in tumor volume (relative to initial volume) for individual mice following 31 days of continuous treatment with the indicated drugs.

(G and H) Correlation between ERBB3 levels and response to the combination of MEK and dual EGFR/ERBB2 inhibitors.

(G) Western blot analysis of ERBB3 and HSP90 levels in a panel of *KRAS* mutant CRC and NSCLC cell lines. HSP90 served as a loading control. ERBB3-high and ERBB3-low cell lines are color coded as black and red, respectively.

(H) High ERBB3 expression correlates with high sensitivity to the treatment containing dual EGFR/ERBB2 inhibitor (afatinib) and MEK inhibitor (trametinib). Sensitivity of each cell line to the combination treatment was presented as synergy score that is calculated based on Lehár et al. (2009).

inhibitors of EGFR and ERBB2, such as afatinib and dacomitinib, did show strong synergy with MEK inhibition. This explains why only the common dimerization partner of these two active complexes was identified in the synthetic lethality screen. Upregulation of RTKs in colon cancer in response to MEK inhibition was also seen by others (Ebi et al., 2011). More specifically, *ERBB3* upregulation as a consequence of MEK inhibitor was seen in *BRAF* mutant thyroid carcinomas and melanomas, but the proposed mechanisms differs from what we observe here (Abel et al., 2013; Montero-Conde et al., 2013).

Due to increased signaling from the active ERBB3 kinase complexes, MEK inhibitors only caused a partial suppression of MEK-ERK signaling in *KRAS* mutant tumors, whereas AKT signaling was even increased in the presence of MEK inhibitors. In contrast, in the presence of both selumetinib and afatinib, MEK-ERK signaling was more completely inhibited, and AKT signaling was also suppressed strongly. We observed a highly synergistic induction of apoptosis when afatinib and selumetinib were combined in *KRAS* mutant colon and lung cancer cells. This may be explained by the finding that the combination of afatinib and selumetinib leads to a more complete inhibition of the phosphorylation of two key inhibitory residues on the proapoptotic BH3-only proteins BAD and BIM. It has been shown previously that phosphorylation of BAD at serine residues 112 and 136 sequesters BAD in 14-3-3 protein complexes at the plasma membrane, thereby inhibiting its proapoptotic action, and a similar model of inhibition by phosphorylation has been proposed for BIM (Datta et al., 1997; Harada et al., 2004; Scheid et al., 1999; Zha et al., 1996). Our data are consistent with a model in which selumetinib and afatinib synergize to unleash the proapoptotic activity of BAD and BIM, resulting in cell death. A similar conclusion was

reached by others (Corcoran et al., 2013). It is possible that cooperative induction of apoptosis through ERK inhibition also underlies the greater efficacy of the combination of BRAF and MEK inhibitors for the treatment of *BRAF* mutant melanoma (Flaherty et al., 2012). Whether the combination therapy we identify here will be successful in the clinic will depend to a large extent on how well the patients tolerate this drug combination.

EXPERIMENTAL PROCEDURES

Synthetic Lethality shRNA Screen

A kinome-centered shRNA library targeting 535 human kinases and kinase-related genes was assembled from The RNAi Consortium (TRC) human genome-wide shRNA collection (TRCHs1.0). The kinome shRNA library was introduced to H358 cells by lentiviral transduction. Cells stably expressing shRNA were cultured in the presence or absence of selumetinib. The abundance of each shRNA in the pooled samples was determined by Illumina deep sequencing. shRNAs prioritized for further analysis were selected by the fold depletion of abundance in selumetinib-treated sample compared with that in untreated sample. Further details are described in Prahallad et al. (2012).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.02.045.

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