Down-regulation of mitogen-inducible gene 6, a negative regulator of EGFR, enhances resistance to MEK inhibition in KRAS mutant cancer cells

Young-Kwang Yoon, Hwang-Phill Kim, Sang-Hyun Song, Sae-Won Han, Do Youn Oh, Seock-Ah Im, Yung-Jue Bang, Tae-You Kim

KRAS proto-oncogene is one of the most frequently mutated oncogenes in human cancer [1,2]. Among the human RAS genes such as HRAS, NRAS, and KRAS, mutations in the KRAS proto-oncogene occur in approximately 35–40% of colorectal cancer (CRC) cases, and consist of single nucleotide point mutations primarily in codons 12 and 13 of exon 2 [3,4]. KRAS mutations lead to deregulation of several effector pathways that control cell proliferation, survival, and migration, thereby promoting malignant transformation [1,5]. The best-characterized RAS effector pathway is the kinase RAF-mitogen activated protein kinase/extracellular signal-regulated kinase (ERK) kinase (MEK) cascade [6,7]. RAF is activated, in part, through RAS-mediated recruitment to the plasma membrane; RAF then activates MEK1/2 which in turn activates ERK1/2. Activated ERK1/2 phosphorylates several cytosolic and nuclear proteins including transcription factors that control the G1-S cell cycle transition [6]. Approximately 30% of human cancers exhibit signs of RAS–ERK signaling pathway activation based on high phosphorylation levels of ERK1/2 [6]. Given the strong genetic evidence implicating the RAS pathway in human cancer etiology, much effort has focused on developing therapeutic agents that inhibit critical downstream pathway components such as RAF and MEK kinases [8–10].

ATP-competitive RAF kinase inhibitors have potent antitumor effects on BRAF mutant (V600E) cancers but are not effective against RAS mutant cancers despite the fact that RAF functions as a key effector downstream of RAS and upstream of MEK [11,12]. Recent studies revealed that RAF inhibitors activate MEK–ERK signaling pathway by inducing BRAF–CRAF hetero-dimerization and enhance tumor growth in KRAS mutant cancers [13–15]. In contrast, the proliferation of BRAF mutant cancers is strongly affected by MEK inhibition whereas KRAS mutant cancers exhibit a more varied response and are generally less sensitive to MEK inhibition [16]. However, the molecular mechanism responsible for this variable expression of KRAS mutant cancers is currently unknown.

Net signal output by receptor tyrosine kinases (RTKs) depends on the dynamic equilibrium between signal generation (positive signaling) and signal attenuation (negative signaling) [17,18]. Recent studies identified several negative regulatory proteins of ErbB receptors such as MIG6, c-Cbl, LRIG1, and Nrdp1 [19–23]. These studies suggest that full oncogenic signaling by RTKs requires the suppression of negative regulators. Furthermore, the loss of negative regulatory proteins may enhance receptor gene amplification to create an environment that promotes receptor overexpression.

MIG6 (mitogen-induced gene6; also known as ERRF11) has been...
identified as an immediate early response gene encoding a non-kinase scaffolding adaptor protein and a negative regulatory protein that inhibit the kinase domains of EGFR and HER2 [19,24–32]. MIG6 expression is generally triggered by EGFR signaling via the RAS–ERK signaling pathway [26,33]. Constitutive overexpression of MIG6 markedly inhibits the mitogenic and transforming activity of EGFR [19,26,34]. Conversely, loss of MIG6 expression enhances cancer cell proliferation and increases herceptin resistance in HER2-amplified breast cancer [25,28]. These data indicate that MIG6 expression is involved in the suppression of ErbB receptor activity. Additionally, these results imply that MIG6 activity may, in part, play a necessary role in a negative feedback loop involving ErbB receptor activity even in KRAS mutant cancer cells.

Our previous study showed that KRAS mutant cancer cells were differentially responsive to AZD6244 via differential activation of EGFR/ALK signaling pathway [35]. To expand our understanding of MEK inhibition in KRAS mutant cancer cells, we conducted cDNA expression array using 4 KRAS mutant cancer cells with differential sensitivity to AZD6244. We found that the heterogeneous responses of KRAS mutant cancer cells to AZD6244 resulted from activation of EGFR/ALK due to inhibition of negative feedback loop via MIG6. These results provide a rational basis for choosing which inhibitor to combine with a MEK inhibitor in KRAS mutant cancer cells.

2. Materials and methods

2.1. Mutational information of EGFR, KRAS, and PIK3CA

Genomic DNA was extracted from two colorectal cancer cell lines (LOVO and HCT116) and two gastric cancer cell lines (SNL668 and AGS). EGFR (exon 18–24) and KRAS (exon 1–2) were then sequenced using previously described primers and methods [35]. All sequencing reactions were conducted in both the forward and reverse directions. All mutations were confirmed at least twice from independent polymerase chain reaction isolates as previously described [35]. Mutational information for P3KCA in each cell line was acquired from the Cancer Genome Project Database of the Wellcome Trust Sanger Institute (www.sanger.ac.uk).

2.2. Cell culture and reagents

Human cancer cell lines LOVO, HCT116, and AGS were purchased from the American Type Culture Collection (Manassas, VA). SNL668 cancer cell line was obtained from the Korea Cell Line Bank (Seoul, Korea) [36]. The cell lines were grown at 37°C in an atmosphere of 5% CO2 in RPMI-1640 containing 10% fetal bovine serum (HyClone, Logan, UT). Lapatinib (an EGFR tyrosine kinase inhibitor), AZD6244 (a MEK inhibitor), U0126 (a MEK inhibitor), and bortezomib (a proteasomal inhibitor) were purchased from Selleck Chemicals (Houston, TX). BKM120 (a pan-PI3K inhibitor) was provided from Novartis. Stock solutions (10 μM/μL) were prepared in dimethyl sulfoxide (DMSO) and stored at −20°C. Lapatinib, AZD6244, U0126, bortezomib, and BKM120 were diluted in fresh medium prior to each experiment, and the final concentration of DMSO was less than 0.1%. Epidermal growth factor (EGF) was purchased from Sigma–Aldrich (St. Louis, MO).

2.3. Antibodies and Western blotting

Cultured cells were washed in ice-cold phosphate buffered saline and lysed in RIPA buffer as previously described [37]. Samples containing equal quantities of total proteins were resolved using sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The resolved proteins were transferred to nitrocellulose membranes and probed with antibodies against pan-EGFR (pY-1068), EGFR, p-ALK (p5473), AKT, p-ERK (pThr-202/Tyr-204), ERK, p-RSK (pS-380), RSK, MIG6 (all from Cell Signaling Technology, Beverley, MA), and α-tubulin (from Sigma–Aldrich, St. Louis, MO). Detection of bound antibodies was conducted using an enhanced chemiluminescence system (Amersham Pharmacia Biotech, Piscataway, Nj).

2.4. Short interfering RNA (siRNA) knockdown and cDNA overexpression

siRNA against MIG6 was purchased from Qiagen (Valencia, CA). Full-length MIG6 cDNA (BC025337; 21C Frontier Human Gene Bank, South Korea) was subcloned into pcDNA3.1+ (Invitrogen, Basel, Switzerland). Cells were transfected with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) in accordance with the manufacturer’s instructions.

2.5. Cell growth inhibition assay

Tetrazolium-dye (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide [MTT]; Sigma–Aldrich, St. Louis, MO) assays were used to evaluate the growth inhibitory effects of AZD6244, lapatinib, BKM120, or AZD6244 plus lapatinib or AZD6244 plus BKM120. Cells (3 × 104) were seeded in 96-well plates, incubated for 24 h, and then treated for 72 h with the drugs at 37°C. After the drug treatment, MTT solution was added to each well and incubated for 4 h at 37°C prior to removing the medium. DMSO was then added and mixed thoroughly for 30 min at room temperature. Cell viability was determined by measuring the absorbance at 540 nm in a VersaMax™ microplate reader ( Molecular Devices, Sunnyvale, CA). The drug concentrations required to inhibit cell growth by 50% were determined via interpolation from dose–response curves using CalcuSyn software (Biosoft, Ferguson, MO). Six replicate wells were utilized for each analysis, and at least three independent experiments were conducted. The data from replicate wells are presented as the mean number of the remaining cells with 95% confidence intervals (CI).

2.6. Analysis of drug combination effects (isobologram analysis)

Analyses of drug interactions were performed by constructing an envelope of additivity using the isobologram method of Steel and Peckham as previously described [38]. Based on available dose–response curves, the combined effects of AZD6244 and lapatinib were analyzed at IC50. Three isoeffect curves were drawn using the following techniques:

2.6.1. Mode I line

When the dose of drug A is chosen, an increment of an effect produced by drug B remains. If two drugs were to act independently, addition is performed by taking the increase in dose, starting from 0, which produces log survival that adds up to IC50 (heteroaddition).

2.6.2. Mode II (A) line

When the dose of drug A is chosen, an isoeffect curve can also be calculated by taking the dose increment of drug B that appropriately contributes to the total effect up to the limit, in this case IC50 (isoaddition).

2.6.3. Mode II (B) line

Similarly, when the dose of drug B is chosen, an isoeffect curve can be calculated by taking the dose increment of drug A that appropriately contributes to the total effect up to IC50 (isoaddition). With a combination of graded doses of drug A and a chosen dose of drug B, a single dose–response curve can be drawn. When the experimental IC50 concentration of this drug combination falls left of the envelope, the two drugs have a supra-additive (synergistic) interaction. When the experimental data point is within the envelope, the combination is considered to be non-interactive. Finally, when the data point is located in the area to the right of the envelope, the combination is considered to be antagonistic. Actual IC50 values were obtained from growth inhibition curves generated after the cancer cells were exposed to a variety of concentrations of AZD6244, lapatinib alone, or a combination of the two drugs. When used in combination, the two drugs were simultaneous applied for 72 h.

2.7. Microarray analysis

Total RNA was extracted from AZD6244-treated for 12 h and DMSO-treated LOVO, HCT116, SNL668, and AGS cells using an RNeasy mini kit (Qiagen, Valencia, CA) in accordance with the manufacturer’s instructions. An Affymetrix Genechip HG-U133 set (Affymetrix, Santa Clara, CA) via DNA Link (Seoul, South Korea) was used for hybridization. All samples were analyzed and reported according to MIAME guidelines. The GeneExpress Software System Fold Change Analysis tool was used to identify all genes with at least 2-fold changes in expression in AZD6244-treated cells compared to the DMSO-treated cells. For each gene fragment, the ratio of the geometric means of the expression intensities in the DMSO-treated and AZD6244-treated cells was calculated, and the fold change was then calculated on a per fragment basis. Confidence limits were calculated using a two-sided Welch modified t-test for the difference of the means for the logs of the intensities.

2.8. Reverse transcription-PCR and real-time PCR analysis

Expression of specific mRNA molecules was semi-quantitated via reverse transcription (RT-PCR) or real-time PCR with gene-specific primers using the iCycler IQ detection system (Bio-Rad Laboratories, Hercules, CA) and SYBR Green I (Molecular Probe, Eugene, OR) in triplicate reactions using previously described methods [39].

2.9. Statistical analysis

Statistical significance of the results was calculated by an unpaired Student’s t-test, and P-values < 0.05 were considered to be statistically significant.
3. Results

3.1. KRAS mutant cancer cells show the heterogeneous response to MEK inhibition

To compare MEK-dependence among the KRAS mutant cancer cells, we first screened the genetic status of each cell line (Fig. 1A). All cell lines (LOVO, HCT116, SNU668, and AGS) contained wild-type EGFR but mutant KRAS. Genetic alterations of KRAS and PIK3CA occurred concurrently in the HCT116 and AGS cell lines.

After characterizing the genetic status, we determined the dependence of these cell lines on MEK for proliferation using AZD6244 (Fig. 1A, Table 1). Cell lines were classified as sensitive (<0.4 μmol/L) or resistant (>0.4 μmol/L) based on the clinically relevant concentration of the drug. The growth inhibition assay showed that LOVO and SNU668 cells were highly dependent on MEK for proliferation. However, HCT116 and AGS cells were resistant to MEK inhibition. To confirm that PI3 KCA mutation mediated resistance to MEK inhibition, we treated AZD6244 with BKM 120, a pan-PI3K inhibitor in HCT116 and AGS cells (Fig. 1B). We found that addition of BKM 120 (1 μmol/L) in the HCT116 and AGS cells made them sensitive to AZD6244. Similar to previous studies [40–42], this result indicated that PI3K–AKT pathway activation via concurrent PIK3CA and RAS mutations may mediate resistance to MEK inhibition.

3.2. Treatment-induced expression of MIG6 is differentially regulated at both transcriptional and post-transcriptional levels in KRAS mutant cancer cells

Previous studies have found that KRAS mutant cancers exhibit variable responses to MEK inhibition due to PI3K–AKT pathway activation via not only concurrent PIK3CA and RAS mutations but also activation of EGFR due to inhibition of negative feedback loop [35,40–43]. By extension, we conducted a set of oligonucleotide microarray experiments to identify specific markers in KRAS mutant cancer cells. We examined global changes in gene expression in AZD6244-sensitive LOVO and SNU668 cells and AZD6244-resistant HCT116 and AGS cells after treatment with 1 μmol/L of AZD6244 for 12 h. Using a filtering strategy to identify the same genes with differential expression following treatment with AZD6244 in sensitive and resistant cells, we found four genes: ERRFI1 (ERBB receptor feedback inhibitor 1; also called MIG6), MAP3K5 (mitogen-activated protein kinase kinase kinase 5), MEF2C (myocyte enhancer factor 2c), and DUSP10 (dual specificity phosphatase 10) (Table 1). The expression of ERRFI1, MAP3K5, and MEF2C was increased as the result of AZD6244 treatment in sensitive cells, but was reduced in resistant cells. Conversely, DUSP 10 expression was reduced following AZD6244 treatment in sensitive cells, but was increased in resistant cells. Among the four genes, we further tested the roles of ERRFI1 gene because the expression was most strongly changed (Table 1). First, we confirmed the change of

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**Fig. 1.** AZD6244 sensitivity varies among different KRAS mutant cancer cell lines. (A) The 4 KRAS mutant cancer cell lines were treated with increasing concentrations of AZD6244 (0, 0.01, 0.1, 1, and 10 μmol/L) for 72 h, and cell viability using MTT was determined by measuring the absorbance at 540 nm in a microplate reader. The dashed (—) lines indicated the clinically relevant concentration of AZD6244. Mutational information was obtained from direct sequencing (EGFR and KRAS) or the Cancer Genome Project Database of the Wellcome Trust Sanger Institute (PIK3CA); (B) HCT116 and AGS cell lines were treated with increasing concentrations of AZD6244 (0, 0.01, 0.1, 1, and 10 μmol/L) with BKM120 (1 μmol/L) for 72 h, and cell viability using MTT was determined by measuring the absorbance at 540 nm in a microplate reader. The dashed (—) lines indicated the clinically relevant concentration of AZD6244.

**Table 1**

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>LOVO</th>
<th>SNU668</th>
<th>HCT116</th>
<th>AGS</th>
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<tbody>
<tr>
<td>IC50 of AZD6244 (μmol/L)</td>
<td>0.011</td>
<td>0.034</td>
<td>0.873</td>
<td>0.959</td>
</tr>
<tr>
<td>Fold expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERRFI1 (MIG6): ERBB receptor feedback inhibitor 1</td>
<td>4.41</td>
<td>4.72</td>
<td>0.20</td>
<td>0.21</td>
</tr>
<tr>
<td>MAP3K5: mitogen-activated protein kinase kinase kinase 5</td>
<td>3.25</td>
<td>3.32</td>
<td>0.43</td>
<td>0.44</td>
</tr>
<tr>
<td>MEF2C: myocyte enhancer factor 2c</td>
<td>4.11</td>
<td>2.73</td>
<td>0.50</td>
<td>0.46</td>
</tr>
<tr>
<td>DUSP10: dual specificity phosphatase 10</td>
<td>0.30</td>
<td>0.38</td>
<td>2.43</td>
<td>2.13</td>
</tr>
</tbody>
</table>
MIG6 (Mitogen-inducible gene 6) expression using RT-PCR (Fig. 2A and B) and Western blotting (Fig. 2C). To further determine whether degradation of MIG6 by AZD6244 was in proteasome-dependent manner, we conducted Western blotting using bortezomib, a proteasomal inhibitor (Fig. 2D). We found that in serum-free conditions with AZD6244 treatment for 3 h resulted in decreased MIG6 expression. However, MIG6 expression was stabilized by inhibition of proteasome activity. In serum-free conditions with EGF treatment for 30 m, the effects of AZD6244 and bortezomib were still observed to a more clear degree. Collectively, down-regulation of MIG6 expression was mediated at both transcriptional (Fig. 2A, B) and post-transcriptional (Fig. 2C and D) levels. Next, we used U0126, the other MEK inhibitor to confirm whether the differential regulation of expression levels of MIG6 specifically

Fig. 2. The down-regulation of MIG6 expression in response to MEK inhibition results in EGFR and AKT activation in MEK inhibitor-resistant cells. (A) The cells were treated with AZD6244 (0.01, 0.1, and 1 μmol/L) for 24 h. MIG6 mRNA levels were assessed using RT-PCR. β-actin served as a loading control. (B) The cells were treated with AZD6244 (0.01, 0.1, and 1 μmol/L) for 24 h. MIG6 mRNA levels were assessed using quantitative real-time RT-PCR. β-actin served as a loading control. Columns represent the mean of three replicates within this experiments; bars indicate ± SE. *P values < 0.05 and **P values < 0.01 for DMSO versus AZD6244 treatment at 0.01, 0.1, and 1 μmol/L. (C) LOVO, SNU668, HCT116, and AGS cells were exposed to the indicated concentrations of AZD6244 for 48 h. The cell lysates were subjected to Western blotting with the indicated antibodies. (D) HCT116 cells were incubated in serum-free medium for 24 h and then treated with AZD6244 (1 μmol/L), bortezomib (1 μmol/L), or AZD6244 plus bortezomib (1 μmol/L, respectively) for 3 h. After the drugs treatment, cells were incubated without or with 50 ng/mL of EGF for 30 m, and lysates were immunoblotted with the indicated antibodies.
was seen for AZD6244. We observed that the effect of U0126 was similar to the effect of AZD6244 (Supplementary Fig. 1).

3.3. The change of MIG6 expression affects AZD6244 sensitivity to KRAS mutant cancer cells

In order to understand the role of MIG6 expression in KRAS mutant cancer cells, we next assessed the phosphorylation states of EGFR, ERK, and AKT after 48 h of treatment with increasing doses of AZD6244 (0, 0.01, 0.1, and 1.0 μmol/L) (Fig. 2C). As anticipated, ERK phosphorylation was inhibited in the presence of AZD6244 in a concentration-dependent manner in both sensitive and resistant cells. In contrast, EGFR and AKT phosphorylation was increased by AZD6244 treatment in resistant cells, but was reduced in sensitive cells. Conversely, MIG6 expression was decreased in response to MEK inhibition in resistant cells, but was increased in sensitive cells. This observation was consistent with the hypothesis that MIG6 may act as a compensatory mediator between MEK/ERK and EGFR/AKT signaling pathways.

To further determine whether the change of MIG6 expression affects the response of sensitive cells to AZD6244, MIG6 was first depleted in AZD6244-sensitive LOVO and SNU668 cells by RNAi knockdown, and the effects on signaling pathway and AZD6244 treatment responses was examined (Fig. 3A). As shown in Fig. 3A, MIG6-specific siRNA efficiently reduced MIG6 protein levels in LOVO and SNU668 cells compared with the non-targeting control. This reduction in MIG6 led to an increase in EGFR and AKT phosphorylation, resulting in resistance to MEK inhibition in LOVO and SNU668 cells. We next expressed either pcDNA3.1, or MIG6 constructs in AZD6244-resistant HCT116 and AGS cells. Unlike control, MIG6 overexpression resulted in decreased EGFR and AKT phosphorylation and re-sensitized HCT116 and AGS cells to MEK inhibition (Fig. 3B). These results indicated that changes in MIG6 expression induced by AZD6244 were highly correlated with AZD6244 sensitivity.

3.4. Synergistic anti-proliferative effect of MEK and EGFR inhibitors is mediated via the blockade of EGFR activation in KRAS mutant cancer cells

Given the activation of EGFR due to inhibition of negative feedback loop via MIG6 in HCT116 cells, we further investigated that whether inhibition of this activation by an EGFR inhibitor is synergized to AZD6244 in HCT116 cells. We first treated HCT116 cells with the EGFR inhibitor, lapatinib in the presence or absence of AZD6244. We found that in serum-free conditions with AZD6244 treatment for 3 h, EGF (50 ng/mL) for 30 min resulted in decreased MIG6 expression followed by dramatic activation of EGFR and AKT (Fig. 4A). Although EGFR and AKT activation was completely abolished by treatment with lapatinib, MIG6 expression was not completely decreased by the addition of lapatinib compared with AZD6244 treatment alone (Fig. 4A). This phenomenon might be due to serum-free conditions with EGF treatment. In medium containing 10% serum, the effects of AZD6244 and lapatinib were still observed to a more clear degree (Fig. 4B). Interestingly, MIG6 expression was maintained by treatment with lapatinib, suggesting that the addition of lapatinib was effective to inhibit EGFR activation via direct targeting of EGFR tyrosine residue and/or additional modulation of MIG6 expression in KRAS mutant cancer cells. These findings suggested that dual inhibition of the EGFR and MEK signaling pathways effects KRAS mutant cancer cells showing activation of EGFR and AKT.

To further validate the additive effects of MEK and EGFR inhibitors, a multiple drug effect analysis was conducted using HCT116 cells (Fig. 4C). The drug concentrations utilized for the isobologram method ranged between 0.01 and 1.0 μmol/L for AZD6244, and between 0.1 and 10 μmol/L for lapatinib. In vitro, the two agents were found to have profound synergistic interactions in HCT116 cells. Even treatment with 0.1 μmol/L of AZD6244, which is below the reported clinically relevant dose in humans, resulted in significantly enhanced the anti-proliferative effects of lapatinib.
These results confirmed that combined treatment with AZD6244 and lapatinib synergistically enhanced the anti-proliferative effects via modulation of MIG6 expression and EGFR/AKT phosphorylation. Furthermore, this provides a rational for treating KRAS mutant cancer cells using a combinatorial strategy.

4. Discussion

The RAS–RAF–ERK signaling pathway is an attractive target for anti-gastrointestinal cancer therapies due to the high activation levels of this pathway via KRAS or BRAF mutations [1,6]. Although AZD6244 can effectively inhibit this pathway, this drug has low activity in gastrointestinal cancers when used as a single agent [16,44,45]. Previous studies have shown that while BRAF mutant cancers are very sensitive to MEK inhibition, RAS mutant cancers exhibit a more heterogeneous response to pharmacologic MEK inhibitors [16]. This variable response can be mediated by simultaneous mutations in RAS and PIK3CA or PTEN [46–48]. Understanding these genetic alterations has promoted the development of drugs that are selective for activating signal pathways and led to establishing more potent combinatorial approaches.

Previously, we showed that dual inhibition of MEK and EGFR or MEK and STAT3 is effective against KRAS mutant cancer cells with PIK3CA or PTEN mutations [35,37]. We also found that EGFR/AKT or STAT3 activation in response to MEK inhibition mediates resistance of KRAS mutant cancer cells to AZD6244. Similarly, a previous study found that a negative feedback loop that activates AKT...
in response to MEK inhibition acts in an EGFR-dependent manner [40]. Taken together, it appears that AKT activation can be achieved by not only PIK3CA activating mutations but also activation of EGFR due to inhibition of negative feedback loop in KRAS mutant cancer cells. To further explore the molecular mechanism underlying this negative feedback activation, we performed a gene expression analysis in four different KRAS mutant cancer cell lines.

Initial testing of the AZD6244 effects on growth and survival led to the identification of two KRAS mutant cancer cell groups, AZD6244-sensitive and AZD6244-resistant, based on AZD6244 C50 values (400 nmol/L). Analysis of gene expression levels identified MIG6 as a mediator between MEK/ERK and EGFR/AKT signaling pathways. Western blotting analysis showed that changes in MIG6 expression induced by AZD6244 treatment were highly correlated with EGFR and AKT activation. Knockdown or restoration of MIG6 expression in sensitive and resistant cells affected signal pathway activation and the response to AZD6244. These results provide a rationale for using a combination of MEK and EGFR inhibitors to treat KRAS mutant cancer cells, and even in cells with a concurrent PIK3CA mutation. Indeed, Western blot and multiple drug effect analyses showed that inhibition of this negative activation of EGFR by lapatinib sensitizes AZD6244-resistant HCT116 cells to MEK inhibition.

Our study showed that EGFR/AKT is activated via the down-regulation of MIG6 expression by the treatment with AZD6244, resulting in resistance to MEK inhibition. However, we note that the induction mechanism of MIG6 expression in response to MEK inhibition is still unknown. The induction of MIG6 expression is primarily mediated by the Ras-RAF-ERK pathway [32]. However, we found that MIG6 expression increased in MEK-sensitive cells although ERK phosphorylation was inhibited by treatment with AZD6244 (Fig. 2). This finding suggests that ERK activation does not have an absolute correlation with the induction of MIG6 expression. Some reports have found that the induction of MIG6 may be mediated by the p38 kinase pathway in isolated hepatocytes [49], and MIG6 induction also occurs through actin-coactivator of the myocardin-related transcription factor [13]-serum response factor (SRF) [50]. After discovering the role for MIG6 in EGFR/AKT activation in KRAS mutant cancer cells, it become important to further determine the mechanism through which MIG6 exerts its effects in cancer cells under different pathophysiological and genetic situations.

The present study provides a molecular-based explanation for the variable response of KRAS mutant cancer cells to MEK inhibition. The results further underscore the need to account for EGFR/AKT activation via the down-regulation of MIG6 expression to achieve optimal cancer therapy treatment responses. Our findings provide a strong rationale for the combined use of MEK and EGFR inhibitors to treat KRAS mutant cancers in a clinical setting.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.canlet.2011.10.023.

References