STAT3 Mediates Resistance to MEK Inhibitor through MicroRNA miR-17

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Abstract

AZD6244 is a small molecule inhibitor of the MEK (MAP/ERK kinase) pathway currently in clinical trials. However, the mechanisms mediating intrinsic resistance to MEK inhibition are not fully characterized. To define molecular mechanisms of MEK inhibitor resistance, we analyzed responses of 38 lung cancer cell lines following AZD6244 treatment and their genome-wide gene expression profiles and identified a panel of genes correlated with sensitivity or resistance to AZD6244 treatment. In particular, ingenuity pathway analysis revealed that activation of the STAT3 pathway was associated with MEK inhibitor resistance. Inhibition of this pathway by JSI-124, a STAT3-specific small molecule inhibitor, or with STAT3-specific siRNA sensitized lung cancer cells to AZD6244 and induced apoptosis. Moreover, combining a STAT3 inhibitor with AZD6244 induced expression of BIM and PARP cleavage, whereas activation of the STAT3 pathway inhibited BIM expression and elicited resistance to MEK inhibitors. We found that the STAT3-regulated microRNA miR-17 played a critical role in MEK inhibitor resistance, such that miR-17 inhibition sensitized resistant cells to AZD6244 by inducing BIM and PARP cleavage. Together, these results indicated that STAT3-mediated overexpression of miR-17 blocked BIM expression and caused resistance to AZD6244. Our findings suggest novel approaches to overcome resistance to MEK inhibitors by combining AZD6244 with STAT3 or miR-17 inhibitors. Cancer Res; 71(10); 3658–68. ©2011 AACR.

Introduction

The MEK (MAP/ERK kinase)/extracellular signal–regulated kinase (ERK) pathway is a critical downstream signal-transduction cascade of most growth factor receptors and is essential for cell growth, survival, differentiation, and transformation. Consequently, MEK inhibitors have been actively investigated in clinical trials for the treatment of various solid tumors (1, 2). Promising results in disease stabilization with the use of tolerable doses of MEK inhibitors have been observed in some groups of patients with lung, pancreatic, or colon cancers or with melanoma (3, 4).

Although MEK inhibitor treatment has produced clinical responses in some patients, a subset of tumors is resistant to this agent, indicating the presence of intrinsic resistance or sensitivity to MEK inhibition. Some reports have shown that BRAF mutations, especially the BRAF (V600E) mutation, are correlated with sensitivity to MEK inhibitors in melanoma and other cancer cells (5, 6). Other studies have indicated that mutations in MEK1 or activation of the phosphatidylinositol 3-kinase (PI3K) pathway due to mutations in the PI3K p110-α catalytic subunit, epidermal growth factor receptor (EGFR), or phosphatase and tensin homolog (PTEN) predict resistance to the MEK inhibitor in KRAS-mutated cells (7–11). However, the molecular mechanism of MEK inhibitor resistance has not been fully elucidated. Specific genetic mutations are good predictors of sensitivity to MEK inhibitors; however, some tumor growth may not totally depend on signaling caused by a specific genetic mutation but rather on multiple signaling pathways. More and more evidence indicates that activation of functionally redundant pathways is responsible for resistance to targeted therapy and that identifying and cotargeting those pathways may overcome resistance and induce synergistic antitumor effects (12). Recently, we and other groups found that a high level of AKT activity is associated with resistance to the MEK inhibitor AZD6244 in lung cancer and that simultaneous inhibition of the AKT and ERK pathways induced increased antitumor activity by AZD6244 (8, 13). Therefore, identifying the signaling pathway associated with MEK inhibitor resistance will help determine the biomarkers that predict responses to MEK inhibitor treatment and develop potential combination strategies to overcome resistance.

In this study, we used genome-wide gene expression profiling followed by Ingenuity Pathway Analysis (IPA) and found...
that activation of the STAT3 pathway was associated with AZD6244 resistance. Thus, inhibition of the STAT3 pathway sensitized resistant cells to AZD6244 treatment, whereas activation of STAT3 induced resistance to AZD6244 in sensitive cells. We further observed that STAT3-mediated MEK inhibitor resistance occurred through microRNA (miRNA) miR-17. Our results suggest that miR-17, which is regulated by the STAT3 pathway, mediated MEK inhibitor resistance by suppressing BIM expression.

Material and Methods

Chemicals and reagents
AZD6244 was synthesized in Dr. William Bornmann’s laboratory at The University of Texas MD Anderson Cancer Center. Antibodies against Janus kinase 1 (JAK1), interleukin-6 signal transducer (IL6ST), phosphorylated STAT3 (p-STAT3), phosphorylated JAK1 (p-JAK1), phosphorylated ERK (p-ERK), phosphorylated Akt (p-AKT), phosphorylated JNK (p-JNK), phosphorylated p38 MAPK (p-p38 MAPK), Akt, ERK, and PARP were purchased from Cell Signaling Technology. Antibodies against BIM were obtained from Calbiochem. Protease inhibitor cocktail, β-actin antibody, and sulfonhydrobamine B (SRB) were obtained from Sigma Chemical Corporation. Plasmid pcDNA3–miR-17 was from Dr. Joshua Mendell (Johns Hopkins University School of Medicine, Baltimore, MD). MiR-17 inhibitor, anti–miR-17, and anti-miRNA negative control were purchased from Ambion. Reagents for the real-time quantitative PCR (qPCR) assay of miR-17 were purchased from Qiagen. Plasmid pcDNA-3.1-STAT3-CA was from Dr. James E. Darnell (The Rockefeller University, New York; ref. 14). STAT3 siRNA was designed on the basis of work by Konnikova and colleagues and synthesized by Qiagen (15).

Cell lines
All human lung cancer cell lines were from Hamon Center for Therapeutic Oncology Research, The University of Texas Southwestern Medical Center, Dallas. Cell lines were maintained in high-glucose RPMI 1640 supplemented with 5% FBS, the cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂ and 95% air.

Cell viability assay
The inhibitory effects of AZD6244 on cell growth were determined using the MTS (Promega) or SRB colorimetric assay, as described previously (16, 17). Each experiment was carried out in octuplicate and repeated at least 3 times. The relative cell viability (%) was calculated using the equation OD₅ /ODC × 100%, where OD₅ represents the absorbance of the treatment group and ODC represents the absorbance of the control group. Median inhibitory concentration (IC₅₀) values were determined using in-house software (DIVISA) and plotted in dose-response curves.

Microarrays
Five micrograms of RNAs were labeled and hybridized to Affymetrix GeneChips HG-U133A and HG-U133B according to the manufacturer’s protocol (http://www.affymetrix.com). Array data were preprocessed with MAS5 (Affymetrix algorithm for probe summarization) and then quantile normalized and log transformed.

Plasmids and anti-miRNA transfection
Lipofectamine 2000 (Invitrogen) was used for the transfection of plasmid DNA or anti-miRNA inhibitor. Forty-eight hours after transfection, cells were treated with AZD6244 for response and apoptosis analysis, or cell lysates and total RNA were prepared for Western blotting and real-time PCR.

Real-time qPCR
Total RNA was isolated from cultured cells using TRIzol reagent (Invitrogen). For analysis of JAK1 and IL6ST and BIM, real-time qPCR was done using the First-Strand Reverse Transcription Kit (Invitrogen) followed by the Real Time PCR Kit from Bio-Rad in accordance with the manufacturer’s protocol. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as the internal control. For miRNA expression analysis, real-time qPCR was done using the miRNA reverse transcription kit (Qiagen) and miRNA assays kit (Qiagen) following the manufacturer’s protocols. miRNA SNORD44 served as an internal control. The sequences of the primers used were as follows: JAK1, 5' primer (5'-GAATGACGCCACACTGACTG-3') and 3' primer (5'-GATGACAAGATGTCCCTCCG-3'); IL6ST, 5' primer (5'-GATGACAAGATGTCCCTCCG-3') and 3' primer (5'-AAA GGACAGGATGTCCCG-3'); and GAPDH, 5' primer (5'-ATC CCATCACCACACTCAG-3') and 3' primer (5'-ATGAGTCC TTCCAGATACC-3').

siRNA knockdown experiments
Transfection was carried out using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Forty-eight hours after transfection, the cells were treated with AZD6244 for the dose-response assay and cell cycle analysis. Cell lysates and RNA were prepared for further analysis by Western blotting and real-time qPCR.

Western blotting analysis
Standard Western blotting was carried out using antibodies described above in the reagent section and as described previously (17). The immunoreactive bands were visualized with the Odyssey Imager (Li-COR Biosciences).

Cell cycle and apoptosis assay
Our cell cycle and apoptosis assays have been described previously (13). The cells were treated with AZD6244 alone or in combination with JSI-124 for 48 hours and harvested by trypsinization. Cells were analyzed on an EPICS Profile II flow cytometer (Coulter Corp.) using the Multicycle AV software (Phoenix Flow Systems). Experiments were repeated at least 3 times.

Pathway analysis
The panel of genes significantly correlated with resistance or sensitivity (P < 0.05) to the MEK inhibitor were imported into IPA (Ingenuity Systems, http://www.ingenuity.com) to analyze network interactions. Networks of these significantly
correlated genes were then algorithmically generated on the basis of their connectivity.

Animal study
Female BALB/c nude mice (6- to 8-weeks old) were purchased from Charles River Laboratories. Animal experiments were carried out according to the protocol (11-03-09933) approval by the MD Anderson institutional review board and in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health. A total of $2 \times 10^6$ H460 cells were inoculated subcutaneously into the right dorsal flanks of the nude mice. Treatments were started when tumors reached an average volume of about 0.1 cm$^3$, which was around 7 days after cells were inoculated. Mice were randomly divided into control and treatment groups ($n = 5$ animals per group). The treatment groups were administered 20 mg/kg AZD6244, 1 mg/kg JSI-124, or combination of AZD6244 (20 mg/kg) and JSI-124 (1 mg/kg), all of which had been solubilized in a medium containing 0.5% hydroxypropyl methylcellulose and 0.1% polysorbate buffer. AZD6244 was administered once daily by oral gavage. JSI-124 was administered intraperitoneally. The control group received the buffer alone. Tumor size was measured by calipers every other day. The tumor volume was calculated with the formula: length $\times$ width$^2$ $\times$ 0.52. Mice were euthanized when their tumor volume was larger than 2000 mm$^3$.

Immunohistochemical staining
Formalin-fixed, paraffin-embedded tissue sections of animal tumor tissue specimens were used for immunostaining with antibodies against p-ERK and p-STAT3 with AEC immunohistochemical staining kit (Invitrogen) following the manufacturer’s instructions.

Statistical analyses
Association between gene expression and responses to treatment with MEK inhibitor was determined by calculating log$_2$ ratios of resistant versus sensitive cell lines, and its significance was determined using Student’s $t$ test. Ingenuity software was used to carry out signaling pathway analysis. Pathways linked with correlated genes were identified as described in the pathway analysis part. The significance of the in vivo animal study data was determined by using the Mann–Whitney $U$ test.

Results
Gene expression profiling identified that activation of the STAT pathway correlated with AZD6244 resistance in lung cancer cell lines
To determine the molecular mechanism underlying MEK inhibitor resistance, previously we have tested responses to AZD6244 in 38 non–small cell lung cancer (NSCLC) cell lines. Susceptibility to AZD6244 differed dramatically between the cell lines, with IC$_{50}$ values ranging from 0.1 to 250 $\mu$mol/L, indicating that human lung cancer cells have a range of degrees of intrinsic resistance or sensitivity to MEK inhibitor treatment (18). Analysis of genetic gene mutations of the cell lines did not find correlation between the gene mutation status of EGFR, KRAS, BRAF, or PI3K and the sensitivity to AZD6244 (18). We then analyzed the gene expression profiles and MEK inhibitor responses of the 5 cell lines that were the most sensitive (Calu-6, H2087, H596, H2073, and H2887), whose IC$_{50}$ values ranged from 0.0287 to 0.519 $\mu$mol/L, and of the 12 most resistant cell lines (H441, H1650, H1819, H1993, HCC366, H460, HCC1359, H1155, H1299, HCC15, HCC95, and HCC2450) whose IC$_{50}$ values ranged from 50 to more than 100 $\mu$mol/L. Through this analysis, we identified many genes whose expression levels correlated strongly (with normal $P < 0.05$) with their sensitivity or resistance to AZD6244 (Fig. 1A and Supplementary Table 1). Further pathway analysis of those genes showed that activation of the STAT3 pathway correlated most strongly with resistance to AZD6244 (Fig. 1B).

Validate the correlation of gene expression with sensitivity to AZD6244
To confirm that activation of the STAT3 pathway was correlated with MEK inhibitor resistance, real-time PCR was carried out to determine the expression of JAK1 and IL6ST, which are related to STAT pathways and were higher in resistant cell lines than that in sensitive cell lines, as determined by our gene expression mRNA array. We checked the expression of these molecules in randomly selected sensitive and resistant cell lines (4 each) using real-time qPCR and Western blotting. Real-time qPCR results showed that expression of both JAK1 and IL6ST was much higher in resistant cell lines than in sensitive cell lines (Fig. 2A and B). Western blotting also confirmed that levels of p-STAT3 and IL6ST were higher in resistant cell lines than in sensitive cell lines, indicating that the STAT3 pathway was constitutively activated in resistant cell lines (Fig. 2C). Based on the database of Cancer Cell line Project (http://www.sanger.ac.uk/genetics/CGP/CellLines/), we did not find the correlation of EGFR, BRAF, and KRAS mutation with sensitivity to AZD6244 (data not shown).

STAT3 pathway regulates sensitivity of lung cancer cells to MEK inhibitor treatment
To further test if STAT3 pathway mediated MEK inhibitor resistance, STAT3 was knocked down in resistant cell lines H460 and H226 (Fig. 3A), and the constitutively active form of STAT3 was overexpressed in 2 AZD6244-sensitive cell lines Calu6 and H1437 (Fig. 3B). However we did not observe changes in JAK1 and IL6ST expression in the cells with overexpression of the constitutively active form of STAT3. Both p-STAT3 and total STAT3 were upregulated in H460 cells after treatment with AZD6244 at time points up to 72 hours (Fig. 3C). SRB assay were carried out to assess the responses to AZD6244 in cells with STAT3 knockdown or overexpression. The results showed that knockdown of STAT3 in H460 and H226 cells significantly sensitized the cells to AZD6244 (Fig. 3D). Knockdown of STAT3 decreased IC$_{50}$ values from more than 50 $\mu$mol/L to less than 1 $\mu$mol/L in both the H460 and H226 cell lines. Activation of the STAT3 pathway in 2 sensitive cell lines, Calu6 and H1437, with the constitutively active form of STAT3 induced resistance to
Figure 1. Pathway analysis identified that the STAT pathway was associated with resistance to the MEK inhibitor AZD6244. A, heat map shows the correlation of gene expression with sensitivity to AZD6244. B, the signaling pathway associated with sensitivity or resistance to AZD6244 obtained by using Ingenuity software analysis. STAT pathway is showed in the map to be associated with MEK inhibitor resistance.
AZD6244 (Fig. 3E). These results suggested that exogenous activation of the STAT3 pathway caused MEK inhibitor resistance in lung cancer cells.

**STAT3 inhibitor JSI-124 sensitized lung cancer cell to AZD6244 in vitro and in vivo**

To determine whether combining AZD6244 with a small molecule STAT3 inhibitor could overcome MEK inhibitor resistance, we tested inhibition of p-STAT3 in H460 cells with different doses of JSI-124, a STAT3 inhibitor (19), and results showed that 50 nmol/L JSI-124 inhibited p-STAT3 levels by approximately 80% (Fig. 4A). Furthermore, inhibition of the STAT3 pathway with 20 and 40 nmol/L JSI-124 significantly sensitized the resistant cell lines H460 and H226 to AZD6244 (P < 0.05; Fig. 4B and C). We also tested several other resistant cell lines, including H2882, HCC827, HCC193, and HCC515, and in all cell lines tested, treatment with a very low concentration of JSI-124 (40 nmol/L) sensitized cells to AZD6244 (data not shown). To confirm these results in vivo, a study was carried out in a mouse human xenograft lung tumor model derived from the H460 cell lines. Combination treatment with AZD6244 and JSI-124 significantly inhibited tumor growth compared to treatment with each drug alone (Fig. 4D and E). We also observed that JSI-124 alone has some effect on the tumor growth inhibition, which was consistent with a previous report (19). Immunohistochemical staining further confirmed that p-ERK and p-STAT3 were significantly inhibited in animal tumor tissue specimens treated with AZD6244, JSI-124, or both compared with that treated with vehicle, indicating that both targets of AZD6244 and JSI-124 can be effectively inhibited with the combination treatment in vivo (Fig. 4F).

**Combination of JSI-124 and AZD6244 induced cell apoptosis through BIM**

To further analyze how JSI-124 sensitized the resistant cells to AZD6244 treatment, cell cycle analysis was carried out. The results showed that a substantial amount of cell apoptosis was induced by combination treatment with AZD6244 and JSI-124 and not by treatment with AZD6244 alone (Fig. 5A). JSI-124 (50 nmol/L) had no effect on the levels of p-AKT, p-JNK, or pp38MAPK (Fig. 5B). Previous studies indicated that induction of BIM expression is a critical step in MEK inhibitor–induced cell apoptosis (20–22). To determine whether apoptosis induction by combination treatment with JSI-124 and AZD6244 is also due to BIM induction, Western blotting was carried out to assess BIM expression in cells treated with AZD6244 alone or combination with JSI-124. The results showed that neither AZD6244 nor JSI-124 alone induced BIM expression in H460 cells, whereas combination treatment with both dramatically induced BIM expression, including BIM-EL, BIM-L, and BIM-SL, in the H460 resistant cell line (Fig. 5C). BIM-EL in particular showed the greatest upregulation in cells with combination treatment. Results also showed that combination treatment with JSI-124 and AZD6244 induced cleavage of PARP, the protein marker for cell apoptosis. However, there was no induction of PARP cleavage in cells treated with AZD6244 alone and only minimal induction of PARP cleavage in cells treated with JSI-124 alone (Fig. 5C). In addition, activation of the STAT3 pathway inhibited the induction of BIM and PARP cleavage by AZD6244 in sensitive Calu6 cells (Fig. 5D). These results indicated that activation of the STAT3 pathway overcame resistance to AZD6244 and
induced cell apoptosis through inducing BIM expression. BIM is regulated by FoxO3A at the transcriptional level (23). ERK also directly phosphorylates BIM at S69 and promotes its degradation (24). However, inhibition of ERK alone in MEK inhibitor-resistant cells could only induce minor BIM expression in AZD6244 resistant cells, although p-FoxO3A was inhibited (Fig. 5E). Inhibition of STAT3 alone had no effect on the phosphorylation of FoxO3A and BIM (Fig. 5E) or any effect on nuclear translocation of FoxO3A (Supplementary Fig. 1) suggesting that regulation of BIM by the STAT3 pathway may not be through FoxO3A or phosphorylation of BIM.

**STAT3 regulates BIM through miR-17**

The results above showed that blocking the STAT3 pathway sensitized resistant cells to AZD6244 by inducing cell apoptosis through BIM. However, how STAT3 cooperated with the ERK-regulating BIM gene is unclear. One recent study found that STAT3 regulated the expression of the miRNA cluster miR-17-92 on the transcriptional level (25). Moreover, studies with transgenic animal models indicated that miR-17-92 promotes cell proliferation and induces tumorigenicity through targeting BIM expression. Thus, we hypothesized that STAT3-mediated MEK inhibitor resistance might occur through the upregulation of miR-17-92, which suppressed BIM by targeting its 3′-untranslated region.

To test this hypothesis, real-time qPCR was carried out to determine miR-17 expression in Calu6 and H1437 cells that had overexpression of the constitutively active form STAT3 and its expression in H460 and H226 cells with STAT3 knockdown. The results of real-time PCR showed that overexpression of constitutively active STAT3 upregulated miR-17 in Calu6 and H1437 cells, whereas knockdown of STAT3 expression in H460 and H226 cells downregulated the expression of miR-17 (Fig. 6A). Consistent with the levels of miR-17 in cells with STAT3 activation, BIM was downregulated at the mRNA level (Fig. 6B), whereas inhibition of miR-17 with anti-miR-17 upregulated BIM RNA in resistant cells (Fig. 6B). To further test whether miR-17 upregulated by STAT3 plays a role in MEK inhibitor resistance, Calu6 and H1437 cells were transfected with miR-17 expression vector, then treated with AZD6244 and assessed by SRB assay. The result showed that overexpression of miR-17 in Calu6 and H1437 cells induced resistance to AZD6244 (Fig. 6C). As it was expected, inhibition of miR-17 with anti-miR-17 combined with AZD6244 induced expression of BIM and PARP cleavage in MEK inhibitor-resistant
Discussion

In this study we tested the MEK inhibitor AZD6244 on a panel of 38 NSCLC cell lines that have been characterized with respect to gene copy number, gene expression, mutation, and protein expression profiles. In our analysis of gene expression profiles, we found one group of genes correlated with MEK inhibitor resistance and another group of genes correlated with MEK inhibitor sensitivity. Analyzing the genes that were significantly correlated with sensitivity or resistance to MEK inhibitors using IPA software, we identified that activation of the STAT3 pathway was associated with MEK inhibitor resistance.

Recently, using a similar gene expression profiling approach, Dry and colleagues also identified that higher levels of IL6 correlated with resistance to MEK inhibition, indicating that the STAT3 pathway may mediate AZD6244 resistance (26). Although we did not find IL6 to be one of the genes

H460 cells (Fig. 6F). These results indicated that miR-17 regulated by the STAT3 pathway plays an important role in the response of lung cancer to MEK inhibitor treatment.
correlated with MEK inhibitor resistance in our study, JAK1, IL6ST, and LIMO4, which are related to JAK-STAT3 pathways, were correlated with MEK inhibitor resistance. JAK1 and IL6ST are molecules directly upstream of STAT3. Overexpression of JAK1 and IL6ST can directly activate STAT3 (27, 28). LIMO4 can bind to and activate IL6ST, thus activating the STAT3 pathway (29). Higher levels of JAK1, IL6ST, and LIMO4 might at least partly contribute to the STAT3 activation and thus induce MEK inhibitor resistance. Another recent study by Yoon and colleagues showed that feedback activation of STAT3 by MEK inhibitor in the KRAS mutated lung cancer cells results in MEK inhibitor resistance, also indicating agreement with our study (30). We further confirmed that inhibition of the STAT3 pathway with STAT3-specific siRNA, or with JSI-124, a STAT3-specific inhibitor sensitized lung cancer cells to MEK inhibitor treatment in vitro and in vivo.

The STAT3 pathway has been shown to be activated in many types of cancer and is associated with cancer transformation, angiogenesis, invasion, and metastasis and with immune system suppression (31, 32). In this study, we found that the combination of AZD6244 and JSI-124 induced cell apoptosis through inducing dramatic BIM expression and PARP cleavage, whereas activation of the STAT3 pathway by overexpression of constitutively active STAT3 in the sensitive cell lines blocked BIM expression and apoptosis induction. Induction of BIM by simultaneous inhibition of the ERK and STAT3 pathways is consistent with previous reports that induction of BIM expression is required for tumor suppression mediated by MEK inhibitors (20, 33). BIM is regulated by both
the AKT and MAPK pathways on the transcriptional level through FoxO3A (23, 34–36). ERK also can directly phosphor-
ylate BIM and thereby promote its degradation (37, 38). However our results showed that JSI-124, a STAT3 inhibitor,
has no effect on p-FoxO3A or p-BIM suggesting that STAT3
regulates BIM by other mechanisms.

Recent studies have shown that BIM was regulated not only
at the transcriptional and protein levels but also at the
posttranscriptional level. Several studies have indicated that
miR-17 promotes tumorigenicity by inhibiting cell apoptosis
through targeting BIM and PTEN (39–41). Importantly, miR-
17 has been reported to be highly expressed in lung cancer and
to promote the proliferation of cancer cells (42) and to be
regulated by STAT3 (25). Recent studies have indicated that
miRNAs are also involved in resistance to chemotherapeutic
agents and possibly to target therapeutic agents as well (43,
44). In this study we found that upregulation of miR-17-92 by
activation of the STAT3 pathway induced MEK inhibitor
resistance, whereas simultaneous inhibition of the MEK
and STAT3 pathways or miR-17 significantly sensitized resis-
tant cells to AZD6244 treatment by upregulating BIM. Our
results not only provide insight into the molecular mechanism
of MEK inhibitor resistance but also indicate novel alternative
approaches for overcoming the MEK inhibitor resistance by
combining AZD6244 with miRNA inhibitors. Given that
STAT3 inhibitors have serious adverse effects, small-molecule
RNA-based miRNA inhibitors have the advantage of less
toxicity and will be promising in future cancer treatment

Figure 6. STAT3 regulates BIM through miR-17. A, STAT3 regulates mir-17 expression in lung cancer cells. A, real-time qPCR analysis of mature
miR-17 expression. B, real-time qPCR analysis of BIM expression. *, P < 0.05, indicates a significant difference. C, overexpression of the miR-17-92 cluster
induced resistance to AZD6244. Calu6 and H1437 cells were transfected with miR-17-92 expression vector. Then, 24 hours after transfection, cells
were treated with AZD6244 at different doses for another 72 hours, and the SRB assay was carried out to analyze cell viability. D, inhibition of miR-17 sensitized
cells to AZD6244. H460 and H226 cells were transfected with anti–miR-17 or negative control. Then, 24 hours after transfection, cells were treated with
AZD6244 with different doses for another 72 hours and the SRB assay carried out to analyze cell viability. E, overexpression of the miR-17-92 cluster blocked
BIM induction by AZD6244. Calu6 cells were transfected with miR-17-92 expression vector or control vector. Then, 48 hours after transfection, cells
were treated with AZD6244 at different doses as indicated for another 48 hours. Western blotting was carried out to analyze the expression of BIM and PARP.
F, inhibition of miR-17 induced BIM expression by AZD6244. H460 cells were transfected with anti-miR-17 or negative control. Then, 48 hours after
transfection, cells were treated with AZD6244 for 48 hours, and Western blotting was carried out with specific antibodies as indicated.

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either as single agents or in combination with other therapeu-
tic drugs.

In summary, using biochemical and biological methods we
identified that the activation of STAT3 pathways mediates
MEK inhibitor resistance. We further found that STAT3-
mediated MEK inhibitor resistance occurs through the inhibi-
tion of BIM by miRNA-17. Our results suggest that the
combination of a small molecule–based inhibitor with a
STAT3 inhibitor or a miR-17 inhibitor may be a productive
approach for lung cancer therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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