Targeted inhibition of the extracellular signal-regulated kinase kinase pathway with AZD6244 (ARRY-142886) in the treatment of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is a common malignancy in Asia and Africa. We previously reported that overexpression of extracellular signal-regulated kinase (ERK) kinase 1/2 (MEK1/2) and ERK1/2 was detected in HCC, and that their activation was required for liver cancer cell proliferation and survival. In the present study, we determined the efficacy of a specific MEK1/2 inhibitor AZD6244 (ARRAY-142886) in treatment of HCC. Treatment of primary HCC cells with AZD6244 led to growth inhibition, elevation of the cleavage of caspase-3 and caspase-7, and cleaved poly(ADP)ribose polymerase, but inhibition of ERK1/2 and p90RSK phosphorylation. Studying the protein expression profile of seven HCC xenografts revealed that their growth rate was positively correlated with the levels of phosphorylated MEK. AZD6244, when given p.o. to mice bearing these xenografts, resulted in a dose-dependent inhibition of tumor growth. AZD6244-induced growth suppression was associated with inactivation of ERK1/2 and p90RSK, and up-regulation of activated caspase-3 and caspase-7, and cleaved poly(ADP)ribose polymerase. Our data suggest that the MEK-ERK pathway plays an important role in the growth and survival of liver cancer cells and that the HCC xenograft models are excellent tools for screening preclinical drugs. Targeted inhibition of the MEK-ERK pathway with AZD6244 may represent an alternative approach for the treatment of this disease. [Mol Cancer Ther 2007;6(1):138–46]
treatment of human HepG2 and Hep3B cells with the MEK1/2-specific inhibitor U0126 led to growth inhibition and apoptosis. Overexpression of activated MEK1 in HepG2 resulted in up-regulation of cyclin D1 and an increase in growth rate (27). Thus, MEK1/2 represents an excellent target for pharmacologic intervention in HCC.

The benzimidazole ARRY-142886 (AZD6244) has been reported to be highly potent MEK inhibitor, with an IC50 of 12 nmol/L against purified MEK (15). AZD6244 is an oral, non-ATP competitive inhibitor and highly specific for MEK1/2, a key enzyme in the Ras-Raf-MEK-ERK pathway. AZD6244 had minimal effects on the p38, c-Jun-NH2-kinase, phosphatidylinositol 3-kinase, and MEK5/ERK5 pathways (28). AZD6244 inhibits both basal and induced levels of ERK1/2 phosphorylation in numerous cancer cell lines with an IC50 as low as 8 nmol/L. It has also shown efficacy in numerous tumor models (29). Sustained inhibition of ERK activity in tumors can be achieved at a dose of 10 mg/kg/d in xenograft studies in mice (28). AZD6244 is currently in phase I clinical trials.

In this study, we show that treatment of primary HCC cells with AZD6244 leads to a time- and dose-dependent reduction in cell proliferation and apoptosis. P.o. delivery of AZD6244 to mice bearing HCC xenografts resulted in dose-dependent growth suppression. The data suggest that blocking MEK-ERK activity with AZD6244 may represent a novel approach to the treatment of HCC.

Materials and Methods

Reagents
AZD6244 (AstraZeneca, Macclesfield, United Kingdom) was dissolved in DMSO to a final concentration of 20 mmol/L and stored frozen under light-protected conditions at −20°C. Antibodies against activated MEK1/2 (20 kDa), cleaved caspase-3, phosphorylated MEK1/2 at Ser217/221, phosphorylated MEK1 (Thr202/Tyr204), p90RSK, phosphorylated p90RSK at Ser359/363, phosphorylated p90RSK at Ser380, phosphorylated p90RSK at SerThr573, and cleaved poly(ADP)ribose polymerase (PARP; 89 kDa) were obtained from Cell Signaling Technology (Beverly, MA). Anti–B-Raf antibody, myelin basic protein (MBP), and recombinant ERK-1 were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Antibodies against MEK1, ERK1, and α-tubulin were from Cell Signaling Technology (Beverly, MA). Anti–B-Raf antibody, myelin basic protein (MBP), and recombinant ERK-1 were from Upstate (Charlottesville, VA). Antibodies against cleaved caspase-7 (20 kDa), cleaved caspase-3, phosphoERK at Thr202/Tyr204, p90RSK, phosphorylated p90RSK at Ser359/363, phosphorylated p90RSK at Ser380, phosphorylated p90RSK at SerThr573, and cleaved poly(ADP)ribose polymerase (PARP; 89 kDa) were obtained from Cell Signaling Technology (Beverly, MA). Anti–B-Raf antibody, myelin basic protein (MBP), and recombinant ERK-1 were from Upstate (Charlottesville, VA). Antibodies against MEK1, ERK1, and α-tubulin were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

HCC Xenografts

The study received ethics board approval at the National Cancer Centre of Singapore as well as the Singapore General Hospital. All mice were maintained according to the ‘Policy on Humane Care and Use of Laboratory Animals’ published by the NIH. The animals were provided with sterilized food and water ad libitum, and housed in negative pressure isolators with 12 h light/dark cycles.

Xenografts were carried out with mice homzygous for the SCID (severe combined immunodeficiency) mutation (SCID/SCID; The Jackson Laboratory, Bar Harbor, ME). Tumors were minced under sterile conditions and fragments that passed through an 18-gauge needle were mixed with RPMI 1640 for transplantation in SCID mice. Seven of eight primary HCCs developed into xenografts. The creation and characterization of these lines of xenografts were previously reported (30).

To investigate the effects of AZD6244 on HCC xenografts, AZD6244 was suspended in water at an appropriate concentration. Mice bearing HCC xenografts were p.o. given, twice a day, with either 100 μL of water (n = 12) or 50 mg (n = 12) or 100 mg (n = 12) of AZD6244 per kilogram of body weight for 21 days, starting from day 7 after tumor implantation. Growth of established tumor xenografts was monitored at least twice weekly by Vernier caliper measurement of the length (a) and width (b) of the tumor. Tumor volume was calculated as (a × b²)/2. Animals were sacrificed 3 h after the last dose of ADZ6244, and body and tumor weights were recorded, with the tumors harvested for analysis.

To study the effects of AZD6244 on caspase-3 activation and MEK1/2 phosphorylation, mice bearing HCC tumors (~800 mm³) were treated with vehicle (n = 4) or 50 mg of AZD6244 per kilogram of body weight (n = 4) for 3 days as described above. Animals were sacrificed 3 h after the last dose, and tumors were harvested and frozen in liquid nitrogen for later analysis. Part of the tumor harvest was fixed in neutral buffer containing 10% formalin for immunohistochemistry.

Immunohistochemical Analysis and Assessment

For immunohistochemical analysis of cleaved caspase-3 and Ki-67, 5-μm sections were cut, dewaxed, rehydrated, and subjected to antigen retrieval as described (14). After blocking endogenous peroxidase activity and reducing nonspecific background staining, the sections were incubated with the primary antibody against cleaved caspase-3 or Ki-67 (overnight at 4°C). Immunohistochemistry was done using the streptavidin-biotin peroxidase complex method, according to the manufacturer’s instructions (Lab Vision, Fremont, CA) using 3,3′-diaminobenzidine as the chromogen. Sections known to stain positively were incubated in each batch and negative controls were also prepared by replacing the primary antibody with preimmune sera. For Ki-67, only nuclear immunoreactivity was considered positive. The labeling index was obtained by counting the number of positive cells among 500 cells. They were expressed as percentage values.

Isolation of Primary HCC Cells

Primary cells from 26-1004, 2-1318, and 4-1318 tumors were isolated separately. The tumors were minced and
washed thrice with modified Eagle’s medium (MEM). The minced tissue was incubated with MEM containing 5% fetal bovine serum and 5 mg/mL of collagenase (Roche Diagnostics Corporation, Indianapolis, IN) at 37°C for 12 h as described previously (31). Cells were harvested by centrifugation at 800 × g for 10 min. The cell pellets were washed thrice with serum-free medium and allowed to grow in growth medium (MEM containing 10% fetal bovine serum).

**Cell Viability and Cell Proliferation**
Primary HCC cells were plated at a density of 2.0 × 10^4 per well in growth medium. After 48 h in growth medium, the cell monolayer was rinsed twice with MEM. Cells were treated with various concentrations of AZD6244 (0, 0.5, 1.0, 2.0, 3.0, and 4.0 μmol/L) for 24 or 48 h. Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (32). Cell proliferation was assayed using a bromodeoxyuridine kit (Roche) as described by the manufacturer. Experiments were repeated at least thrice, and the data were expressed as mean ± SE.

**Detection of Apoptosis**
Primary HCC cells were grown in eight-chamber slides and treated with 0, 0.5, 1.0, 2.0, 3.0, and 4.0 μmol/L of AZD6244 in SRF medium for 24 h. Cells were fixed with PBS containing 4% formalin solution for 1 h at room temperature and washed with PBS. Apoptosis was detected by the terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) assay using the *In situ* Cell Death Detection kit (Roche) as described by the manufacturer. Apoptotic cells were then visualized under a fluorescent microscope equipped with a FITC filter. The labeling index was obtained by counting the number of positive cells among 500 cells per region. They were expressed as percentage values.

**Western Blot Analysis**
Tissue or cells were lysed and subjected to Western blot analysis as previously described (14). Blots were incubated with indicated primary antibodies and 1:7,500 horseradish peroxidase–conjugated secondary antibodies. All primary antibodies were used at a final concentration of 1 μg/mL. The blots were then visualized with a chemiluminescent detection system (Amersham Pharmacia Biotech) as described by the manufacturer.

**Assay of MEK Kinase Activity**
Anti-MEK1 antibody (Santa Cruz Biotechnology) was used to immunoprecipitate MEK1 molecules. MEK kinase activity was measured as the ability of immunopurified MEK1 to activate recombinant ERK1 in a coupled assay using MBP as the end point of the assay (33). Phosphorylated MBP was resolved on a 14% SDS-PAGE gel and vacuum-dried before exposure to X-ray film.

**Statistical Analysis**
For quantitative analysis, the sum of the density of bands corresponding to protein blotting with the antibody under study was calculated and normalized to the amount of α-tubulin. After normalization with α-tubulin, changes in the expression of the protein under study in the treated samples were expressed relative to the basal levels of this protein in the control sample. Differences in cell number and levels of protein under study were analyzed by ANOVA.

**Results**
To study the effects of AZD6244 on proliferation and viability, primary HCC cells were studied *in vitro*. For the time course and dose-response experiments, primary 2-1318 cells were treated with vehicle and escalating doses of AZD6244 for 24 and 48 h. Cell viability and cell growth were assessed by MTT assay and bromodeoxyuridine incorporation, respectively. AZD6244 caused a time- and dose-dependent reduction in DNA synthesis (Fig. 1A) and cell viability (*P* < 0.01; Fig. 1B). Significant inhibition of bromodeoxyuridine incorporation was observed as early as 24 h posttreatment (*P* < 0.01). A 50% reduction in cell viability was seen at a dose of <0.5 μmol/L after 48-h incubation (Fig. 1B). Similar results were obtained when 4-1318 and 26-1004 cells were used.

To investigate whether AZD6244-induced growth arrest and apoptosis in primary HCC cells were associated with the inactivation of ERK, lysates from AZD6244-treated 2-1318 cells were subjected to Western blot analysis. AZD6244 treatment caused a dose-dependent elevation of phosphorylated MEK1/2 at Ser217/221 and MEK1 at Ser218/222; it also caused a loss of ERK1/2 phosphorylation (Fig. 1C). Because MEK phosphorylation is mediated by B-Raf (34), the level of B-Raf was determined. Figure 1C shows that B-Raf expression was increased and was in concordance with MEK phosphorylation. Phosphorylation of p90RSK at Ser389/395 was also inhibited by AZD6244, indicating that ERK kinase activity was inactivated (Fig. 1C). We next investigated whether the apoptotic machinery was activated by AZD6244 treatment. As shown in Fig. 1C, the 89-kDa cleaved PARP fragment was detected in the AZD6244-treated samples. The cleaved forms of caspase-3 were readily detectable at a dose as low as 0.5 μmol/L AZD6244 (Fig. 1C). A cleaved caspase-7 fragment was also observed in concordance with caspase-3 activation (Fig. 1C). Figure 1D shows that the rate of apoptosis in primary 2-1318 cells was significantly increased in a dose-dependent manner by AZD6244 (*P* < 0.01). Similar results were obtained when 4-1318 and 26-1004 cells were used.

To study the time course of ERK1/2 and p90RSK inactivation and caspase activation, primary 2-1318 cells were treated with 1 μmol/L of AZD6244 and then harvested at different times for Western blot analysis. As shown in Fig. 2A, phosphorylated ERK1/2 was significantly decreased at 3 h and barely detectable at 6 h after AZD6244 exposure. Phosphorylation of p90RSK was significantly inhibited at 6 h (Fig. 2A). Cleaved forms of caspase-3, caspase-7, and PARP (Fig. 2A) were clearly detected 6 h after AZD6244 treatment and reached maximum levels at 12 h. Inhibition of caspase activation with Z-VD-FMK completely blocked AZD6244-induced ERK1/2 activation.
and apoptosis in these cells (Fig. 2B), confirming that AZD6244 induced apoptosis in primary 2-1318 cells by activating the caspase pathway.

We previously showed that phosphorylation of MEK1/2 was detectable by immunohistochemistry in 46 of 46 HCCs examined (26), and that activation of MEK-ERK is required for growth and survival of primary HCC cells. To examine the role of MEK-ERK in HCC growth and survival in vivo, we established seven (2-1318, 4-1318, 5-1318, 26-1004, 30-1004, 29-1104, and 2006) HCC xenografts from primary HCC tumors. The establishment and characterization of these xenografts are reported elsewhere (30). To investigate whether the difference in growth rate among the different xenograft lines (Fig. 3A) was associated with activation of MEK1/2, Western blot analysis was done as described in Materials and Methods with cells treated as described above. Blots were incubated with the indicated antibodies and representative samples were shown. Apoptotic cells were determined by TUNEL assay; the rate of apoptosis was expressed as a percentage of the total cells counted. Similar results were obtained when 26-1004 and 4-1318 cells were used. Columns with different letters are significantly different from one another (P < 0.01) as determined by ANOVA. Columns, mean; bars, SE. 

MEK1 at Ser^{218/222} and Thr^{206} were detected in the 4-1318 (lane 5), 2-1318 (lane 7), and 26-1004 (lane 6) lines; on the other hand, low basal levels were detected in the 5-1318 (lane 1), 29-1104 (lane 2), 30-1004 (lane 4), and 2006 (lane 3) lines. Unexpectedly, the basal levels of phosphorylated ERK1/2 (Fig. 3B) and phosphorylated MEK kinase activity (Fig. 3C), as determined by levels of phosphorylated MBP, in the 2-1318, 4-1318, and 26-1004 lines were slightly lower than those observed in the 5-1318, 29-1104, 30-1004, and 2006 lines. Total ERK1/2 and MEK among xenografts were not significantly different among the lines (Fig. 3B). Thus, the level of phosphorylated ERK1/2 was not in accordance with that of phosphorylated MEK1/2.

To examine the role of MEK in the regulation of HCC growth in vivo, mice bearing xenografts that express high (4-1318, 2-1318, and 26-1004) and low (5-1318 and 29-1104) levels of phosphorylated MEK1/2 were treated with inhibitory doses of AZD6244. As shown in Fig. 3D, high basal levels of Cdk-5 and phosphorylated ERK1/2 (Fig. 3B) and phosphorylated MEK kinase activity (Fig. 3C), as determined by levels of phosphorylated MBP, in the 2-1318, 4-1318, and 26-1004 lines were slightly lower than those observed in the 5-1318, 29-1104, 30-1004, and 2006 lines. Total ERK1/2 and MEK among xenografts were not significantly different among the lines (Fig. 3B). Thus, the level of phosphorylated MEK1/2 was not in accordance with that of phosphorylated MEK1/2.
levels of phosphorylated MEK1 were treated with the MEK inhibitor AZD6244. Both animal toxicity and the ability of AZD6244 to inhibit tumor formation and progression were determined. For the dose-response experiment, 4-1318 xenografts were implanted on both sides of male SCID mice. Although a previous study reported that sustained inhibition of ERK activity in tumors could be achieved at a dose of 10 mg/kg/d (28), we were unable to abolish ERK1/2 phosphorylation at that dose in HCC xenografts (data not shown). Therefore, we selected 50 and 100 mg/kg doses of AZD6244 for our in vivo study. Mice bearing the 4-1318 xenografts were given p.o. AZD6244 starting on day 7 after tumor implantation. Tumor formation was 100% in both the control and two AZD6244-treated groups. As shown in Fig. 4A, the growth rate of 4-1318 xenografts was decreased by AZD6244 in a dose-dependent manner ($P < 0.01$). The tumor weight in mice treated with 50 and 100 mg ADZ6244 per kilogram was ~40% and 18% of the controls, respectively (Fig. 4B). At a dose of 100 mg/kg, AZD6244 caused ~10% decrease in body weight, suggesting a serious toxicity. No overt toxicity as measured by weight loss, and morbidity was observed at the dose of 50 mg/kg, suggesting good safety. AZD6244 when given at the dose of 50 mg/kg also significantly suppressed the growth of the 5-1318 and 29-1104 xenografts ($P < 0.01$). For a dose of 50 mg/kg AZD6244, the treated versus control ratios for 4-1318, 2-1318, 26-1004, 5-1318, and 29-1104 xenografts were 0.41, 0.50, 0.31, 0.64, and 0.70, respectively. The difference in sensitivity to AZD6244, as determined by the treated versus control ratio, between high (4-1318, 2-1318, and 26-1004) and low (5-1318 and 29-1104) MEK1 phosphorylation lines was significant at $P < 0.05$. The
We next examined the association between antitumor activity of AZD6244 and its ability to inhibit the MEK-ERK phosphorylation and activity in 4-1318 xenografts. Tumors were harvested on day 21, 3 h after administration of either AZD6244 or vehicle. As shown in Fig. 6, phosphorylated ERK1/2 (Fig. 6A) and MEK kinase activity (Fig. 6B) in 4-1318 tumors obtained from animals treated with 50 mg/kg AZD6244 was significantly decreased compared with vehicle-treated mice (P < 0.01). Similarly, AZD6244 significantly inhibited phosphorylation of ERK1/2 in 2-1318, 5-1318, and 26-1004 (Fig. 6C). No differences were observed for the total ERK1/2 (Fig. 6A and C). As shown in Fig. 6A, the levels of B-Raf and phosphorylated MEK1 at Ser218/222 in AZD6244-treated 4-1318 xenografts were also elevated (P < 0.01), suggesting that AZD6244 activated a signaling pathway upstream of MEK but inhibited MEK activity. Because the 90-kDa ribosomal S6 kinase (p90RSK) is a target of ERK1/2 (25) and phosphorylation of p90RSK at Ser380, Thr359, and Ser363 is important for its activation (35), we wanted to determine if phosphorylation of p90RSK was also inhibited. Figure 6A shows that phosphorylation of p90RSK at Ser380 and Thr359/363 was significantly reduced (P < 0.01) but not completely suppressed by AZD6244. This suggests that, in HCC, phosphorylation of p90RSK is not entirely dependent on ERK1/2 activity, or that only a small amount of ERK1/2 activity is sufficient to phosphorylate quite a lot of p90RSK. The results show that a positive correlation between the antitumor activity of AZD6244 and its ability to inhibit ERK activity exists.

We have shown that treatment of primary HCC cells with the MEK1/2-specific inhibitor AZD6244 resulted in growth inhibition and apoptosis. We investigated whether these phenomena also occurred in vivo. As shown in Fig. 6A, the cleaved 89-kDa PARP fragment was detected in AZD6244-treated xenografts. The cleaved forms of caspase-3 (19 and 17 kDa fragments) and caspase-7 (19 kDa fragment) were also observed in concordance with cleaved PARP (Fig. 6A). Immunohistochemical analysis revealed that the Ki-67 labeling indexes in the vehicle-treated group and 50 mg AZD6244-treated groups were 14.4 ± 3.6% and 4.3 ± 1.8%, respectively. The percentages of cells that stained for cleaved caspase-3 were 0.1 ± 0.1% and 4.5 ± 2.7% for the vehicle-treated group and 50 mg of AZD6244 per kilogram of body weight group, respectively. Similar results were obtained when sections from 2-1318, 26-1004, 29-1104, and 5-1318 xenografts were stained with Ki-67 and cleaved caspase-3 antibodies (data not shown). These results support the view that the reduction in HCC growth and induction of apoptosis by AZD6244 is associated with the inhibition of cell proliferation and caspase-3 activation.

Discussion
Neither the systemic chemotherapy nor hormonal therapy tested has shown an unequivocal benefit in terms of survival in patients with advanced HCC. Thus, there is a significant unmet medical need for the development of effective therapies able to stabilize or slow the progression...
of HCC. The poor efficacy of medical treatment of patients with HCC and the dissatisfaction with such treatment has motivated the development of HCC xenografts to evaluate the efficacy of novel or existing drugs before clinical trials. Through a better understanding of the molecular basis of hepatocarcinogenesis, new preventative and therapeutic modalities are being developed.

In this study, we show that blockage of MEK1/2 activity with AZD6244 results in growth arrest and apoptosis in established HCC in vivo. The growth behavior of HCC xenografts is positively correlated with the levels of phosphorylated MEK1 at Ser218/222 and Thr286. At a dose of 50 mg/kg, AZD6244 does not cause any apparent harm to the animals, but does suppress tumor growth in a dose-dependent manner. Treatment of primary human liver cancer cells with the MEK inhibitor AZD6244, which inhibits the MEK-ERK pathway activation, leads to a time- and dose-dependent reduction in cell proliferation and viability, implicating the requirement of MEK activity for liver cancer cells to proliferate and survive.

The presence of activated MEK in aggressive HCC xenografts suggests that MEK activation may be linked to the malignant progression of liver cancer. It has been
shown that expression of cyclin D1 is controlled by MEK-ERK signaling (36, 37), and expression of activated mitogen-activated protein kinase phosphatase 1, a negative regulator of MEK (38), significantly depresses the ability of these cells to proliferate in response to growth factor stimulation. In the present study, the high levels of phosphorylated MEK observed in fast-growing xenografts may reflect local autocrine/paracrine signaling. Activation of MEK-ERK by autocrine/paracrine growth factors may help the cells survive even in the presence of limited nutrients, and to increase the secretion of angiogenic factors from the tumor cells (39, 40). In vivo, these angiogenic factors then stimulate neovascularization, which is essential for the growth, survival, invasion, and metastasis of liver cancer cells. It is possible that the AZD6244-induced apoptosis in vivo is also related to the inhibition of neovascularization. It has been reported that PD98059 and U0126, two other MEK inhibitors, are able to impede the growth of Ras-transformed cells in soft agar and to reduce the urokinase secretion controlled by growth factors such as epidermal growth factor, transforming growth factor-α, and fibroblast growth factor in an autocrine manner in the squamous cell carcinoma cell lines UM-SCC-1 and MDA-TV-138 (41). It remains to be determined whether AZD6244 is able to control invasive tumor phenotype of HCC cells. Experiments are under way to investigate this possibility.

In mammalian cells, there are at least two pathways involved in apoptosis. One involves caspase-8, which is recruited by the adapter molecule Fas/APO-1–associated death domain protein to death receptors upon extracellular ligand binding (42, 43). The other involves cytochrome c release–dependent activation of caspase-9 through Apaf-1 (44, 45). No changes in either Fas or FasL expression in AZD6244-treated primary HCC cells was observed. We did observe increased cleaved caspase-3, cleaved caspase-7, and cleaved PARP in AZD6244-treated cells. It remains to be determined whether AZD6244 also causes the release of cytochrome c, which is responsible for the activation of both caspase-7 and caspase-3.

In the present study, we did not observe a correlation between phosphorylated MEK1 and ERK1/2. The basal levels of phosphorylated ERK1/2 and MEK kinase activity in fast-growing xenografts are slightly lower than slow-growing ones. Given the role of MEK in ERK phosphorylation, it is interesting to observe that three lines of these fast-growing xenografts are slightly lower than slow-growing ones. The present study, in the present study, we also observed that B-Raf and phosphorylated MEK1 at Ser218/222 and Thr286 are elevated in AZD6244-treated xenografts and primary cells. These suggest that AZD6244 also activates a signaling pathway upstream of MEK. Because MEK is a target of B-Raf, it is possible that elevation in phosphorylated MEK but not ERK1/2 in vivo and in vitro following AZD6244 treatment is due to AZD6244-induced up-regulation of B-Raf (Fig. 6A).

Unlike the case with cytotoxic agents, HCC patients responding to treatment with a signal transduction inhibitor such as AZD6244 are likely to share common molecular alterations of the target or pathway, such as Ras or Raf mutations driving activation of the MEK-ERK pathway. The present study shows that the 5-1318 and 29-1104 lines, expressing low levels of phosphorylated MEK1, are relatively less sensitive to AZD6244. This suggests that patients with high levels of activated MEK in their tumors will respond to AZD6244 in a more potent manner than patients who have low levels of activated MEK. Designing clinical trials that accrue patients based on the level of activated MEK in their tumors is therefore a special interest. Analysis of tumor samples for this specific biomarker is important for determining the likely in vivo effects of AZD6244 inhibitor. Here, we have shown that AZD6244 inhibits tumor growth when given alone, suggesting that at least some patients may be amenable to single-agent therapy. As for all potential anticancer drugs, inhibitors of the MEK-ERK pathway may not be very effective as individual therapeutic agents because HCC patients possess more than one genetic defect (15). For maximal therapeutic benefit, it may be necessary to combine AZD6244 with another signal transduction inhibitor or conventional chemotherapeutic drugs such as doxorubicin or 5-fluorouracil. In the meantime, a multitude of other targeted agents have become available that should also test in combination with MEK inhibitors. The elucidation of the optimal combinations can be expected to unfold over time.

We have shown that treatment of HCC cells with the MEK inhibitor AZD6244 leads to growth inhibition and apoptosis in vitro. Using five lines of xenografts, we show that p.o. delivery of AZD6244 leads to growth inhibition of HCC xenografts in vivo and induces apoptosis in liver cancer cells. Our data implicate important role(s) of the activated MEK-ERK pathway in liver cancer cell proliferation and survival. Therefore, targeted inhibition of MEK activity by AZD6244, or other MEK inhibitors such as PD0325901 (15), may represent a highly valuable alternative approach for the treatment of HCC.

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References


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