Sorafenib Potently Inhibits Papillary Thyroid Carcinomas Harboring RET/PTC1 Rearrangement
Ying C. Henderson,1 Soon-Hyun Ahn,1 Ya'an Kang,1 and Gary L. Clayman1,2

Abstract

Purpose: Papillary thyroid carcinomas (PTC) are the most common type of thyroid malignancy with one of the two mutations, RET/PTC rearrangement or BRAF mutation. Both mutations are able to activate the MEK/ERK signaling transduction pathway and result in the activation of transcription factors that regulate cellular proliferation, differentiation, and apoptosis. Sorafenib (Nexavar, BAY 43-9006) is a multikinase inhibitor, and in this study, we tested its effects on PTC cells carrying either mutation.

Experimental Design: The effects of sorafenib on cell proliferation and signaling were evaluated in vitro on PTC cells using growth curves, cell cycle analysis, and immunoblotting. Using an orthotopic mouse model, we determined the antitumor effects of sorafenib in vivo.

Results: The concentration needed for 50% growth inhibition (GI50) by sorafenib was 0.14 μmol/L for the PTC cells with the RET/PTC1 rearrangement, and 2.5 μmol/L for PTC cells with a BRAF mutation, both readily achievable serum concentrations. After 3 weeks of oral administration of sorafenib (80 mg/kg/d) in mice, small (94% reduction compared with controls) or no tumor growth was detected in mice inoculated with PTC cells bearing the RET/PTC1 rearrangement, whereas the tumor volume of the orthotopic tumor implants of PTC cells with a BRAF mutation was reduced 53% to 54% (as compared with controls).

Conclusions: PTC cells carrying the RET/PTC1 rearrangement were more sensitive to sorafenib than PTC cells carrying a BRAF mutation. Because RET/PTC rearrangements are unique to thyroid carcinomas, our findings support the clinical evaluation of sorafenib for patients with PTC and the identification of patients most likely to respond to sorafenib treatment.

The most common type of thyroid malignancy is papillary thyroid carcinoma (PTC). Most PTCs carry one of the two mutations: RET/PTC rearrangements (12 different RET/PTC rearrangements have been reported, including RET/PTC1, RET/PTC2, and RET/PTC3 rearrangement being studied most) or BRAF mutations (1–6). All RET/PTC rearrangements result in constitutive activation of RET tyrosine kinase (7–10). The most common BRAF mutation in cancer is a thymine to adenine substitution at codon 600 (V600E) of the BRAF protein in exon 15 (5, 11). This mutation in BRAF results in constitutive activation of BRAF. Either mutation (RET/PTC rearrangement or BRAF mutation) is able to activate the mitogen-activated protein kinase kinase (MEK1/2 or MAPKK) and then MAPK (ERK1/2) signaling transduction pathway, resulting in the activation of a variety of transcription factors that regulate cellular proliferation, differentiation, and apoptosis.

A number of protein kinase inhibitors have been developed and have shown activity in the clinic (12–16). We previously showed that MEK1/2 inhibitors (PD98059 and U0126) can inhibit PTC cell growth in vitro and the expression of phosphorylated ERK1/2 (p-ERK1/2) decreased in PTC cells with the RET/PTC1 rearrangement and with a BRAF mutation after treatment with either PD98059 or U0126 (17). Despite the inhibitory effects of these inhibitors to PTC cells, both PD98059 and U0126 were used for in vitro study only due to the poor solubility of PD98059 and inactivity of U0126 in vivo (14). To expand on these observations, we have evaluated the activity of sorafenib (BAY 43-9006, Nexavar), a multikinase inhibitor being developed by Bayer and Onyx Pharmaceuticals. Sorafenib has been approved for use in humans for the treatment of advanced renal cell carcinoma (18–20) and its activity is being evaluated in additional tumor types including melanoma (21), breast carcinoma (22), thyroid carcinomas (23, 24), and colon cancer (22). Sorafenib is a biaryl urea and has been shown to inhibit the serine/threonine kinase Raf (BRAF and c-Raf) and RET, c-kit, and receptor tyrosine kinases (platelet-derived growth factor receptor and vascular...
endothelial growth factor receptor; refs. 22, 24, 25). In anaplastic thyroid carcinomas with a BRAF mutation, sorafenib was able to inhibit tumor growth in xenografts with the 50% maximal inhibitory concentrations (IC50) being 0.5 to 1 μmol/L (23). In medullary and papillary thyroid carcinomas with RET point mutations, sorafenib inhibited tumor growth in xenografts and IC50 were 49 to 147 nmol/L, depending on the different types of RET point mutations (24). However, sorafenib has not been evaluated for activity in PTC cells with the RET/PTC1 rearrangement in comparison to PTC cells with a BRAF mutation. In this study, we treated PTC cells carrying either BRAF mutation or RET/PTC1 rearrangement with sorafenib. We found that the concentration of sorafenib needed for 50% growth inhibition (GI50) to the PTC cells bearing the RET/PTC1 rearrangement were 18-fold lower than the PTC cells carrying a BRAF mutation. At 1 μmol/L, sorafenib was able to dephosphorylate both MEK1/2 and ERK1/2 in PTC cells with the RET/PTC1 rearrangement. In PTC cells with a BRAF mutation, at least 5 μmol/L of sorafenib was needed to reduce the expression of phosphorylated MEK1/2 (p-MEK1/2) and ERK1/2 (p-ERK1/2). In our orthotopic mouse model for PTC (26), we found that sorafenib inhibited or dramatically reduced the tumor growth (94% reduction) in PTC with the RET/PTC1 rearrangement and moderately reduced the tumor volume of PTC with a BRAF mutation (53-54% reduction) when compared with untreated (vehicle). Our results showed that PTC cells carrying the RET/PTC1 rearrangement were potently inhibited by sorafenib as compared with the PTC cells carrying a BRAF mutation. Because RET/PTC1 rearrangement is a characteristic unique to thyroid carcinoma, sorafenib may have significant therapeutic benefit for patients with recurrent or advanced PTC.

Materials and Methods

Cell lines. PTC cell lines carrying the RET/PTC1 rearrangement (TPC-1) and a BRAF mutation (V600E; NPA87) were kindly provided by Dr. Jerome Hershman (VA Greater Los Angeles Healthcare System, Los Angeles, CA; refs. 27, 28). The cells were maintained in RPMI 1640 (Mediatech, Inc.) containing 10% fetal bovine serum (Hyclone), nonessential amino acid mixture (Cambrex BioScience), 1 mmol/L of sodium pyruvate (Cambrex BioScience), and 2 mmol/L of L-glutamine (Mediatech, Inc.) containing 10% fetal bovine serum. For PTC cells carrying the RET/PTC1 rearrangement, cells were serum-starved for 16 h before treating with sorafenib in RPMI 1640 supplemented with 5% fetal bovine serum. For PTC cells carrying the RET/PTC1 rearrangement, cells were washed with Hank’s buffered salt solution before adding sorafenib. Protein extracts from treated PTC cells were prepared in lysis buffer containing 20 mmol/L of Tris-HCl (pH 7.4), 1% Triton X-100, 300 mmol/L of NaCl, 1 mmol/L of phenylmethylsulfonyl fluoride, 50 mmol/L of NaF, 1 mmol/L of NaVO4, and 1% protease inhibitor cocktail III (Calbiochem). Total protein concentrations were estimated using the Bradford assay (Bio-Rad) with bovine serum albumin as a standard. For Western blot analysis, proteins were resolved on 10% SDS-polyacrylamide gels using a MiniProtein II Electrophoresis System (Bio-Rad). The proteins were then transferred to Hybond-ECL membranes (GE Healthcare Bioscience) using a mini-transblot electrophoretic transfer cell (Bio-Rad) at 80 V for 1 h at room temperature. After transfer, the membranes were blocked and probed with antibodies at 4°C overnight as indicated by the manufacturer. The antibodies for p-MEK1/2, total MEK1/2, p-ERK1/2, total ERK1/2, poly(ADP-ribose) polymerase (PARP), Bcl-2, Bcl-xl, Bax, Bak, and Bad (Cell Signaling Technology) were used at a dilution of 1:1,000 and a monoclonal antibody against actin (Sigma-Aldrich) was used at a dilution of 1:2,000.

Cell cycle analysis. PTC cells (2 × 104) were plated the day before treatment in a 10 cm dish. On the day of treatment, cells were first washed once in RPMI medium without serum. Then sorafenib was added to the cells in RPMI without serum containing 1 mg/mL of fatty acid–free bovine serum albumin after treatment in a 10 cm dish. On the day of treatment, cells were first washed once with 100% ethanol, and stored at 4°C overnight. Propidium iodide (50 μg/mL, 1 mL) containing DNase-free RNase (3.3 mg/mL; Roche Applied Science) was added to the fixed cells, which were then incubated at 37°C for 30 min. Cell cycles were determined by flow cytometry using FACSChannel Fluor Cytometer (BD Biosciences).

Tumor growth in athymic mice using an orthotopic model. The procedures for an orthotopic thyroid carcinoma model in mice were described elsewhere (26). Athymic Ncr-nu/nu mice were obtained from the National Cancer Institute at ages 8 to 12 weeks and housed for at least a week after arrival. All experimental procedures and care for mice were in accordance with the Institutional Animal Care and Use Committee and the Department of Veterinary Medicine of M. D. Anderson Cancer Center. Eight to 10 mice per group were anesthetized subcutaneously with a cocktail (100 μL/10 g body weight of 10 mg/mL ketamine and 1 mg/mL xylazine). PTC cells (1 × 106 cells in 0.8% NaCl solution) were inoculated into the thyroid gland and mice were monitored daily for tumor growth. After 2 to 3 weeks following inoculation, sorafenib [dissolved in Cremophor EL-95% ethanol (50:50) and diluted with water] was given to mice at 40 mg/kg twice daily by oral gavage for 5 consecutive days/wk for 3 weeks. Tumor sizes were measured with calipers at the end of the 3 weeks and tumor volume (V) was calculated with the formula [V = (length × width2) / 2]. For controls, mice were given diluted Cremophor EL-95% ethanol (50:50) alone.

Statistical analysis. Statistical analysis was done using the honest significant difference post hoc test from multifactorial ANOVA by STATISTICA data analysis software system version 7.1 (StatSoft Inc.) for cell growth and cell cycle analysis. The unpaired Student’s t test was used for analysis of tumor volume. P ≤ 0.05 was considered significant.

Results

GI50 of sorafenib in PTC cells. To monitor the effects of sorafenib for the inhibition of PTC cell growth, the concentration exposure was T, the absorbance at time zero was T0, and the control absorbance with DMSO only was C. GI50 was determined using Prism 3.0 (GraphPad Software).

Western blotting analysis. PTC cells with a BRAF mutation were serum-starved for 16 h before treating with sorafenib in RPMI 1640 containing 1 mg/mL of fatty acid–free bovine serum albumin without serum. For PTC cells carrying the RET/PTC1 rearrangement, cells were washed with Hank’s buffered salt solution before adding sorafenib. Protein extracts from treated PTC cells were prepared in lysin buffer containing 20 mmol/L of Tris-HCl (pH 7.4), 1% Triton X-100, 300 mmol/L of NaCl, 1 mmol/L of phenylmethylsulfonyl fluoride, 50 mmol/L of NaF, 1 mmol/L of NaVO4, and 1% protease inhibitor cocktail III (Calbiochem). The proteins were then transferred to Hybond-ECL membranes (GE Healthcare Bioscience) using a mini-transblot electrophoretic transfer cell (Bio-Rad) at 80 V for 1 h at room temperature. After transfer, the membranes were blocked and probed with antibodies at 4°C overnight as indicated by the manufacturer. The antibodies for p-MEK1/2, total MEK1/2, p-ERK1/2, total ERK1/2, poly(ADP-ribose) polymerase (PARP), Bcl-2, Bcl-xl, Bax, Bak, and Bad (Cell Signaling Technology) were used at a dilution of 1:1,000 and a monoclonal antibody against actin (Sigma-Aldrich) was used at a dilution of 1:2,000.

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Results

GI50 of sorafenib in PTC cells. To monitor the effects of sorafenib for the inhibition of PTC cell growth, the concentration...
needed to inhibit 50% cell growth (GI_{50}) was determined in PTC cells (TPC-1 and NPA87). Serial dilutions (1:5) of sorafenib were prepared, starting at 10 μmol/L and going down to 0.64 μmol/L. After 2 days of incubation with varying concentrations of sorafenib, an MTT assay was done. The GI_{50} for PTC cells with the RET/PTC1 rearrangement was 0.14 μmol/L; for PTC cells with a BRAF mutation, it was 2.5 μmol/L, as determined by Prism software.

**Inhibition of cell growth by sorafenib.** After determining the GI_{50} of sorafenib in PTC cells, we treated PTC cells with sorafenib at three different concentrations: 0.005, 0.05, or 0.5 μmol/L for PTC cells with the RET/PTC1 rearrangement (0.036× GI_{50}, 0.36× GI_{50}, or 3.6× GI_{50}, respectively) and 0.15, 1.5, or 5 μmol/L for PTC cells with a BRAF mutation (0.06× GI_{50}, 0.6× GI_{50}, or 2× GI_{50}, respectively). After the PTC cells carrying the RET/PTC1 rearrangement were treated with sorafenib for 4 days, MTT assays revealed that cell growth was reduced by 69% (P < 0.001) at 0.5 μmol/L, 65% (P < 0.001) at 0.05 μmol/L, and 32% (P < 0.001) at 0.005 μmol/L (Fig. 1A). For PTC cells with a BRAF mutation, we observed 78% growth inhibition at 5 μmol/L (P < 0.001) and 51% inhibition at 1.5 μmol/L of sorafenib (P < 0.001; Fig. 1B). No growth inhibition was observed in PTC cells carrying a BRAF mutation when 0.15 μmol/L of sorafenib was used (Fig. 1B). These results show that sorafenib inhibits the growth of PTC cells, and that PTC cells bearing RET/PTC1 rearrangement were significantly more sensitive to sorafenib than their BRAF counterparts.

**Dephosphorylation of MEK1/2 and ERK1/2 in PTC cells by sorafenib.** Because sorafenib is a multikinase inhibitor with activity against both BRAF and RET kinases, the downstream effectors of these kinases are MEK1/2 and ERK1/2. The ability of sorafenib to decrease the phosphorylation of MEK1/2 (p-MEK1/2) and ERK1/2 (p-ERK1/2) was examined in PTC cells. PTC cells carrying the RET/PTC1 rearrangement were treated with different concentrations of sorafenib for 2 h. Protein extracts were prepared and the status of p-MEK1/2 and p-ERK1/2 was evaluated using Western blot analysis. We found that the expression of both p-MEK1/2 and p-ERK1/2 became undetectable at concentrations of ≥1 μmol/L (Fig. 2A). The expression of p-ERK1/2 was undetectable as early as 0.5 h after treating PTC cells with 1 μmol/L of sorafenib as shown on the time course study (Fig. 2A). A limited amount of p-ERK1/2 was detectable after 8 h of treatment with sorafenib. The expression of p-ERK1/2 returned to preexposure levels after the cells had been treated for 24 h. The total ERK1/2 expression remained the same during all treatments.

The effect of sorafenib on the expression of p-MEK1/2 and p-ERK1/2 in PTC cells carrying a BRAF mutation seems to be different than its effects on PTC cells carrying the RET/PTC1 rearrangement. The amount of p-MEK1/2 and p-ERK1/2 decreased when cells were treated at concentrations of ≥5 μmol/L of sorafenib (Fig. 2B). Using 10 μmol/L of sorafenib, the reduction of p-ERK1/2 expression was detected as early as 0.5 h following treatment. The expression of p-ERK1/2 returned to pretreatment levels after treating with 10 μmol/L of sorafenib for 6 h or longer (Fig. 2B).

**PARP cleavage detected in PTC cells with a BRAF mutation after sorafenib treatment.** To determine the mechanism of cell growth inhibition in PTC cells with a BRAF mutation by sorafenib, we evaluated the expression of the caspase 3 substrate PARP as an indicator of apoptosis using Western blot analysis. PTC cells with a BRAF mutation showed cleaved PARP after 2 days of treatment with sorafenib, and the expression remained detectable for up to 4 days (Fig. 2C). For cells with the RET/PTC1 rearrangement, no cleaved PARP was detected even up to 4 days of treatment (data not shown).

To investigate the mechanism of cell growth inhibition in PTC cells carrying the RET/PTC1 rearrangement, a cell cycle analysis was done after the PTC cells were treated with different concentrations of sorafenib for 24 h. For PTC cells carrying the RET/PTC1 rearrangement, >95% of cells were in G1 phase and reached statistical significance (P = 0.006) compared with the amount of cells in G1 phase in untreated cells (76%) when 0.5 or 1.5 μmol/L of sorafenib was used (Fig. 3A). Besides the changes in G1 phase, no significant changes in other phases of the cell cycle (G2-M, S, and apoptotic) were observed. However, no G1 arrest was observed in PTC cells carrying a BRAF mutation after treatment with sorafenib (1.5 or 5 μmol/L) for 24 h (Fig. 3B). These data therefore suggest that for PTC cells with the RET/PTC1 rearrangement the mechanism of growth inhibition was G1 arrest whereas for PTC cells with a BRAF mutation, the mechanism of growth inhibition was apoptosis, as indicated by PARP cleavage.

**Inhibition of tumor growth by sorafenib in an orthotopic mouse model.** The inhibitory effects of sorafenib were tested in vivo using an orthotopic mouse model (26). PTC cells were inoculated into the mouse thyroid. After 2 to 3 weeks, sorafenib was given to these mice (see Materials and Methods) for 3 weeks. At the end of the 3 weeks, tumor sizes were measured and tumor volume was calculated (Table 1). This experiment was done twice and the results of both experiments are shown here (Table 1). In mice inoculated with PTC cells carrying the RET/PTC1 rearrangement following sorafenib...
treatment, no tumor was detected in the first experiment and ~94% reduction in tumor volume was observed in the second experiment, when compared with untreated (vehicle). In mice bearing tumor from PTC cells carrying a BRAF mutation, average tumor volume after sorafenib treatment was reduced 54% for the first experiment and 53% for the second experiment, when compared with untreated mice. To further test the mechanism of tumor suppression by sorafenib, protein extracts were prepared from mice tumors and the expression of cleaved PARP was determined by Western blot analysis (Fig. 2D). Cleaved PARP was detected only in mice inoculated with PTC cells carrying a BRAF mutation and treated with sorafenib. Cleaved PARP was undetectable in both types of untreated mice tumor (RET/PTC1 or BRAF mutated) and in those mice inoculated with the RET/PTC1-rearranged PTC cells and treated with sorafenib. This data correlated well with the data from in vitro studies and suggested that sorafenib was significantly more effective in its inhibition of tumor growth in PTC cells carrying the RET/PTC1 rearrangement than in PTC cells carrying a BRAF mutation.

**Discussion**

In the present study, we evaluated the effects of sorafenib in PTC cells carrying the RET/PTC1 rearrangement or a BRAF mutation. We found that the GI_{50} (The National Cancer Institute renamed the IC_{50} value, the concentration that causes 50% growth inhibition, to the GI_{50} value to emphasize the correction for the cell count at time zero) for PTC cells with the RET/PTC1 rearrangement was much lower than the GI_{50} for the PTC cells with a BRAF mutation (18-fold difference). This inhibition of cellular growth resulted in G1 arrest and seemed to be associated with the dephosphorylation of MEK1/2 and ERK1/2 in PTC cells with the RET/PTC1 rearrangement. In PTC cells with a BRAF mutation, PARP cleavage seemed to be the mechanism of cell growth inhibition.

Our findings regarding the GI_{50} of sorafenib in PTC cells with the RET/PTC1 rearrangement are in agreement with a previous study (24). In that study, the IC_{50} of sorafenib for NIH fibroblasts carrying the RET/PTC3 rearrangement was 47 nmol/L (24). We also found that at low GI_{50} levels (0.036× or 0.36× GI_{50}), sorafenib was able to inhibit 32% or 65% of cell growth in PTC cells with the RET/PTC1 rearrangement, respectively. This was a statistically significant inhibition compared with untreated cells. For cells with a BRAF mutation, the GI_{50} of sorafenib is, in general, in the micromolar range. Although the GI_{50} against the purified BRAF mutant (V600E) protein was only 38 nmol/L (22), this potent activity did not translate into activity in cells. Sorafenib has been tested against several cell lines with activating BRAF mutations, including human melanoma cells (880 nmol/L), human colon carcinoma cells (2-4 µmol/L; ref. 22), anaplastic thyroid carcinoma cells...
Cells in the G1/G0, G2-M, and S phase are shown. The percentage of cells in sub-G1 sorafenib (0.15 mol/L) was detected at 0.5 mol/L or 1.5 and 5 μmol/L sorafenib for 24 h, respectively. Cells not treated with sorafenib (0 μmol/L) were used as a control. After flow cytometry, the percentage of cells in the G1, G0, G2-M, and S phase are shown. The percentage of cells in sub-G1 (apoptotic) phase was too small to show on the graph and was not included.

In searching for an explanation for the different sensitivities of different cells to sorafenib, we examined the effect of sorafenib on the expression of p-MEK1/2 and p-ERK1/2. In PTC cells with the RET/PTC1 rearrangement, the dephosphorylation of MEK1/2 and ERK1/2 was observed at 0.15 μmol/L (0.06 × GI50). These data suggested that PTC cells carrying a BRAF mutation require a higher concentration of sorafenib to inhibit cell growth than in those with the RET/PTC1 rearrangement.

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Table 1. Sorafenib inhibits tumor growth in mice

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Treatment</th>
<th>No. of mice</th>
<th>Average tumor volume (mm³)</th>
<th>95% Confidence interval (mm³)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>RET/PTC1</td>
<td>Vehicle—first time</td>
<td>7</td>
<td>101.5</td>
<td>36.7-166.3</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Sorafenib—first time</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Vehicle—second time</td>
<td>4</td>
<td>701.9</td>
<td>590.7-813.1</td>
<td>&lt;0.001</td>
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<td></td>
<td>Sorafenib—second time</td>
<td>3</td>
<td>45.8</td>
<td>20.3-71.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BRAF</td>
<td>Vehicle—first time</td>
<td>5</td>
<td>252.3</td>
<td>206.0-298.6</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Sorafenib—first time</td>
<td>7</td>
<td>115.7</td>
<td>78.1-153.3</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Vehicle—second time</td>
<td>8</td>
<td>297.3</td>
<td>255.6-339.0</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Sorafenib—second time</td>
<td>8</td>
<td>139.3</td>
<td>115.9-162.7</td>
<td>&lt;0.001</td>
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</table>
we observed PARP cleavage after sorafenib treatment, which implicated the involvement of caspase because PARP is a substrate of caspase 3, we did not detect any change in the Bcl-2 family of proteins, such as Bcl-2, Bcl-xl, Bax, Bak, and Bad (data not shown). Similar PARP cleavage was also detected when PTC cells with a BRAF mutation were treated with MEK1/2 inhibitors (PD98059 and U0126; ref. 17). Yu et al. have also shown that the expression of several Bcl-2 proteins (Bcl-2, Bcl-xl, Bax, Bak, Bid, and Bim) does not change after treatment with 5 μmol/L of sorafenib in a human non–small cell lung carcinoma cell line (29). However, they detected the down-regulation of another member of the Bcl-2 protein family, Mcl-1, in non–small cell lung carcinoma, renal cell carcinoma, colon cancer, breast cancer, Jurkat acute T cell leukemia, cholangiocarcinoma, chronic myelogenous leukemia, and chronic lymphocytic leukemia cells. They showed that sorafenib enhanced proteasome-mediated Mcl-1 degradation. This down-regulation of Mcl-1 resulted in mitochondrial cytochrome c release and activation of caspase and other proapoptotic agents. This finding could explain the case in PTC cells with a BRAF mutation, in which we observed only the PARP cleavage but did not detect a change in the other Bcl-2 proteins tested. Clearly, the effects of sorafenib on cell lines are more complex and the mechanism through which apoptosis is induced varies from cell line to cell line. This might be dependent on the underlying genetic changes supporting the tumor cells’ growth and survival.

After establishing that sorafenib inhibited PTC cell growth in vitro, the effect of sorafenib was tested in vivo. Previous studies for the effects of sorafenib in vivo have been done by inoculating mouse flanks with tumor cells (22–24). These xenograft experiments have shown that sorafenib inhibited tumor growth in vivo. We have developed an orthotopic model in which PTC cells were inoculated directly inside the mouse thyroid (26). To our knowledge, this is the first thyroid orthotopic model to mimic the tumor microenvironment as in the case of thyroid carcinomas occurring in patients. We found that in mice inoculated with PTC cells carrying the RET/PTC1 rearrangement, 0% to 6% tumor growth was detected after sorafenib treatment, as compared with untreated mice. A moderate decrease in tumor volume (53-54% reduction compared with vehicle) was observed in mice inoculated with PTC cells carrying a BRAF mutation. In addition, cleaved PARP was detected only in mice inoculated with PTC cells carrying a BRAF mutation and treated with sorafenib. These data suggest that (a) sorafenib not only inhibited PTC cell growth in vitro but it also inhibited tumor cell growth in vivo, (b) PTC cells with the RET/PTC1 rearrangement were more sensitive to sorafenib treatment in terms of tumor cell growth rate (in vitro) and tumor volume (in vivo), and (c) the mechanism of sorafenib in BRAF-mutated PTC cells might be due to apoptosis induction versus cytostasis in RET/PTC1-rearranged PTC cells. Based on this information, sorafenib should be tested in human clinical trials against PTC with stratification based on the molecular profile.

Many phase I, II, and III trials of sorafenib for different types of cancer are currently under way in this country (19, 20), especially since the approval in 2005 of sorafenib by the U.S. Food and Drug Administration for treating advanced renal cell carcinoma. Our study provides evidence that sorafenib is more potent in PTC cells with the RET/PTC1 rearrangement than in PTC cells with a BRAF mutation. By testing the mutation that occurs in PTC cells from patients (BRAF mutation or RET/PTC rearrangement), we may be able to predict which patients are more likely to respond to sorafenib therapy. Because RET/PTC rearrangement is restricted to PTC, sorafenib may be an ideal drug for selected PTC patients with this type of mutation.

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