Inhibition of Human Tumor Xenograft Growth by Treatment with the Farnesyl Transferase Inhibitor B956

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ABSTRACT

ras oncogenes are present in several types of cancers but are most frequently described in colon and pancreatic carcinomas. Consequently, ras is being targeted for drug development as a means to develop therapies for these types of cancer. The ras protein is posttranslationally modified by the addition of a farnesy group, followed by cleavage of the COOH-terminal 3 amino acids and methylation of the prenylated cysteine. Because the posttranslational addition of farnesy is obligatory not only for the remaining modifications to take place but also for ras control of cell growth, inhibitors of farnesyl transferase are being developed as potential antitumor agents. In this report, a new peptidomimetic inhibitor of farnesyl transferase is described. This compound, B956, and its methyl ester B1086, inhibit the formation of colonies in soft agar of 14 human tumor cell lines expressing different ras oncogenes at concentrations between 0.2 and 60 μM. Higher concentrations of B956 (10—80 μM) were required to inhibit colony formation by 5 tumor cell lines without ras mutations. B956/B1086 at 100 mg/kg also inhibit tumor growth by EJ-1 human bladder carcinoma, HT1080 human fibrosarcoma, and to a lesser extent by HCT116 human colon carcinoma xenografts in nude mice. Furthermore, inhibition of tumor growth by B956 is shown to be correlated with inhibition of ras posttranslational processing in the tumor. Thus, peptidomimetic inhibitors of ras farnesyl transferase have the potential to be developed as therapy for ras-dependent tumors.

INTRODUCTION

The ras proto-oncogene is involved in the control of cell proliferation and differentiation. In normal cells, ras switches between inactive GDP-bound and active GTP-bound states (1). Binding of growth factors like epidermal growth factor or platelet-derived growth factor to their receptors results in the activation of the ras proteins by promoting the exchange of bound GDP for GTP (2). The activated ras, by a still unknown mechanism, triggers a phosphorylation cascade involving ras, mitogen-activated protein kinase kinase, and mitogen-activated protein kinase, and ending in the activation of nuclear transcription factors. The final result is stimulation of cell growth or differentiation. Inactivation of ras occurs when the bound GTP is hydrolyzed to GDP, a reaction stimulated by GTPase-activating protein. The cycle is thus closed, and ras remains in inactive form until a new growth signal arrives (for reviews, see Refs. 3 and 4).

Oncogenic ras protein has lost the ability to switch between the inactive and active states and remains permanently activated. Although there are several ways in which ras can remain activated, the most common seems to be mutations at codons 12, 13, or 61, which though there are several ways in which ras can remain activated, the subsequent modifications, as well as membrane localization and function, of the ras protein (9—12). Therefore, inhibition of farnesyl transferase is being sought by several laboratories as a possible mechanism for inhibition of ras growth (13). In this report, we describe in vivo data obtained with a potent new peptidomimetic inhibitor of farnesyl transferase, B956. This compound inhibits the growth of tumors induced in nude mice by ras-transformed cells, including 3 human xenografts expressing different types of oncogenic ras. B956 also inhibits the ability of several human tumor cell lines to form colonies in soft agar, a common assay for tumorigenicity. Furthermore, it is shown that inhibition of tumor growth is correlated with inhibition of ras posttranslational processing in the tumor. Although differences in sensitivity to the inhibitor were observed in different cell lines, inhibition of ras farnesyl transferase appears promising.

MATERIALS AND METHODS

Cells. NIH 3T3 cells transformed with H-ras (61L) (zh1, or pZIP-ras-F) have been described previously (11). NIH 3T3 cells transformed with K-ras (12V) (DK1) were prepared by infection with retrovirus carrying K-ras 4B (12V) clone. Infected cells were selected with G418, and single clones were isolated, grown, and tested for K-ras expression, growth in soft agar, and tumor formation in nude mice. A single clone (DK1) was chosen for these studies. EJ-1 cells were kindly provided by Dr. C. Der (University of North Carolina, Chapel Hill, NC). The remaining cell lines were obtained from American Type Culture Collection (Rockville, MD) or Dainippon Seiyaku (Osaka, Japan) as indicated in Table 1. All cells were maintained as recommended.

Soft Agar Colony Growth. Cells at the density indicated in Table 1 were seeded on 24-well plates in 0.2 ml of 0.33% Noble agar (DIFCO Laboratories) in tissue culture medium supplemented with serum over 0.4 ml of 0.66% Noble agar in the same culture medium. The top agar layer was overlaid with 0.2 ml of 0.66% Noble agar in culture medium. T24 and MiApaca-2 cells were seeded in 0.2 ml Matrigel (Collaborative, MA.) and covered with an upper layer of Matrigel in culture medium. In all cases, the upper agar or Matrigel layer was covered with 0.2 ml of culture medium supplemented with serum and 2.5% DMSO or 2-fold serial dilutions of B956. After 10—21 days at 37°C, 0.2 ml of 3.3 mg/ml 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide in PBS was added and incubated for 1 h. The number and size of the stained colonies were analyzed with an image analyzer program (Spectrum; Mitani Corp., Fukui, Japan).

Tumor Implantation. Female BALB/c-nu nude mice (5—6 weeks of age; Japan SLC, Inc., Shizuoka, Japan) were housed in barrier facilities on a 12-h light/dark cycle, with food and water ad libitum. Cells (1 × 10^6) EJ-1, 1 × 10^6 HT1080, and 8 × 10^6 HCT116) in 0.1 ml HBSS were injected s.c. into the flank of the animal at day 0. At day 1, mice were separated into control (n = 10) and treatment (n = 5 each) groups. B956 or its methyl ester

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Seiyaku (Osaka, Japan).

were injected i.p. Treatment was started on day 1 at the concentrations and was determined twice weekly. Tumor volume was calculated according to the vehicle. Tumor volume was measured on the days indicated, and body weight treatment schedules indicated in the figure legends. Control animals received B1086 were dissolved in saline containing 2% Tween 80 just before use and by Western blotting with F235—1.7.1 antibody (Oncogene Science).

5 mM Tris-HCl (pH 7.5)-S mM EDTA-1 mM phenylmethylsulfonyl fluoride.

activity in vitro. The concentration to inhibit H-ms farnesylation by 50% is in the nanomolar range (IC50 = 11 nM). In whole cells (zHl or DK1), inhibition of Anchorage-independent Growth of Human Tumor Cell Lines. B956 (Fig. 1) is a new and potent inhibitor of farnesyl protein transferase.

RESULTS

B956 inhibits H- and K-ras posttranslational processing by 50% at 0.5 and 25 µM, respectively. In this report, these studies have been extended to the effect of B956 on anchorage-independent growth of 19 human tumor cell lines, as well as the 2 model cell lines zH1 and DK1. The panel of human tumor cell lines tested is summarized in Table 1, where the presence and identity of ras mutation is indicated. This group of 19 cell lines represents a variety of tissues, as well as ras mutations. Treatment with B956 resulted in inhibition of the number and size of colonies formed. The concentration of B956 that reduced the number of colonies by 50% is shown in Fig. 2. There is a similar inhibition pattern for the formation of colonies in soft agar as that seen for inhibition of ras processing (namely, cell lines expressing different types of ras show differences in sensitivity to the inhibitor). Tumor cell lines expressing H-ras oncogene seem to be the most sensitive, followed by cell lines expressing mutated N-ras. The more resistant tumor cell lines are the ones expressing mutant K-ras as well as cells with ras mutations. The IC50 for inhibition of colony formation among the human cell lines expressing K-ras fluctuates between 1.7 µM for the most sensitive (HCT116) and 50 µM for the least sensitive (HCT15). In contrast, H-ras transformed cell lines are inhibited by submicromolar concentration of B956, whereas for N-ras-transformed cells, 50% inhibition occurs at concentrations around 5 µM B956.

In contrast to other reports (14, 15), transformed cell lines without ras mutations were inhibited in their capacity to form colonies in soft agar at concentrations between 16 and 80 µM B956. Approximately one-half of the K-ras-transformed cell lines tested were inhibited at the same concentrations of B956. We have previously shown a similar result with another inhibitor, B581 (11). This compound at 100 µM inhibited colony formation of NIH 3T3 fibroblasts transformed either with the ras oncogene or mutant H-ras modified by geranylgeranylation or myristylation by about 50% (11). The fact that non-ras-mutated cell lines are inhibited in their colony-forming capacity is not unexpected if other farnesylated proteins, like lamins or other yet unidentified proteins, are considered. However, the involvement of these proteins in the control of cell growth is not clear. In summary, transformants expressing either H- or N-ras oncogenes are very sensitive to inhibition by the FTase inhibitor B956. In contrast, only 50% of K-ras mutant cell lines are inhibited at relatively low (i.e., below 10 µM) concentrations of inhibitor. The remaining 50% of the K-ras-transformed cells tested were inhibited by concentrations of B956 that also inhibit non-ras-mutated cell lines (i.e., between 10 and 100 µM).

Table 1 Human tumor cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tissue of origin</th>
<th>ras mutation (Ref.)</th>
<th>IC50 (µM)</th>
</tr>
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<tbody>
<tr>
<td>H520 (A)</td>
<td>Lung</td>
<td>(27)</td>
<td>2 x 10^3</td>
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<tr>
<td>COL0320DM (D)</td>
<td>Colon</td>
<td>(28)</td>
<td>1 x 10^4</td>
</tr>
<tr>
<td>COL0205 (A)</td>
<td>Colon</td>
<td>(28)</td>
<td>4 x 10^4</td>
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<tr>
<td>HT29 (A)</td>
<td>Colon</td>
<td>(29)</td>
<td>2 x 10^4</td>
</tr>
<tr>
<td>WiDr (D)</td>
<td>Colon</td>
<td>(30)</td>
<td>1 x 10^4</td>
</tr>
<tr>
<td>EJ-1</td>
<td>Bladder</td>
<td>H-12D (31)</td>
<td>1 x 10^4</td>
</tr>
<tr>
<td>T24 (D)</td>
<td>Bladder</td>
<td>H-12D (32)</td>
<td>1 x 10^4</td>
</tr>
<tr>
<td>A-427 (A)</td>
<td>Lung</td>
<td>K-12D (33)</td>
<td>5 x 10^4</td>
</tr>
<tr>
<td>A549 (D)</td>
<td>Lung</td>
<td>K-12S (33)</td>
<td>5 x 10^4</td>
</tr>
<tr>
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<td>Colon</td>
<td>K-12D (34)</td>
<td>4 x 10^4</td>
</tr>
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<td>SW620 (A)</td>
<td>Colon</td>
<td>K-12V (35)</td>
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<td>Colon</td>
<td>K-13D (23)</td>
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<td>HCTI16 (A)</td>
<td>Colon</td>
<td>K-13D (23)</td>
<td>1 x 10^4</td>
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<tr>
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<td>Lung</td>
<td>K-61H (36)</td>
<td>1 x 10^4</td>
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<td>HCT-15 (A)</td>
<td>Colon</td>
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</tr>
<tr>
<td>MIA PaCa-2 (A)</td>
<td>Pancreas</td>
<td>K-12C (38)</td>
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<tr>
<td>HT-1080 (D)</td>
<td>Fibrosarcoma</td>
<td>N-61K (39)</td>
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</tr>
<tr>
<td>THP-1 (A)</td>
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<tr>
<td>HL-60 (A)</td>
<td>Leukemia</td>
<td>N-61L (41)</td>
<td>1 x 10^4</td>
</tr>
</tbody>
</table>

* Origin of cells: A, American Type Culture Collection (Rockville, MD); D, Dainippon Seiyaku (Osaka, Japan).

B1086 were dissolved in saline containing 2% Tween 80 just before use and were injected i.p. Treatment was started on day 1 at the concentrations and treatment schedules indicated in the figure legends. Control animals received vehicle. Tumor volume was measured on the days indicated, and body weight was determined twice weekly. Tumor volume was calculated according to the following equation: tumor volume (mm³) = (Length x Width)²/2.

After 2–3 weeks, mice were sacrificed, and tumor weights were determined. Significance was determined by Student's t test.

Inhibition of ras Posttranslational Processing. Tumors were induced in nude mice by zH1 cells as above. At day 6, when the tumor was visible, B956 was administered i.p. to 3 mice, at a concentration of 200 mg/kg every 12 h for a total of 7 injections. After determining tumor size, the mice were sacrificed, and the tumor was minced and treated with collagenase (1.2 mg/ml) and DNase (0.3 mg/ml). A single cell suspension was obtained by filtration through a nylon mesh. The cells were washed with cold PBS and lysed by sonication in 5 mM Tris-HCl (pH 7.5)-5 mM EDTA-1 mM phenylmethylsulfonyl fluoride.

Cell debris was removed by centrifugation at 12,000 x g for 5 min, followed by cytosol and membrane fractionation by centrifugation at 100,000 x g for 30 min. Protein (50 µg/lane) was separated by SDS-PAGE and ras was identified by Western blotting with F235-1.7.1 antibody (Oncogene Science).

RESULTS

Inhibition of Anchorage-independent Growth of Human Tumor Cell Lines. B956 (Fig. 1) is a new and potent inhibitor of FTase activity in vitro. The concentration to inhibit H-ras farnesylation by 50% is in the nanomolar range (IC50 = 11 nm³). In whole cells (zH1 or DK1), approximately one-half of the K-ras-transformed cell lines tested were inhibited at the same concentrations of B956. We have previously shown a similar result with another inhibitor, B581 (11). This compound at 100 µM inhibited colony formation of NIH 3T3 fibroblasts transformed either with the ras oncogene or mutant H-ras modified by geranylgeranylation or myristylation by about 50% (11). The fact that non-ras-mutated cell lines are inhibited in their colony-forming capacity is not unexpected if other farnesylated proteins, like lamins or other yet unidentified proteins, are considered. However, the involvement of these proteins in the control of cell growth is not clear. In summary, transformants expressing either H- or N-ras oncogenes are very sensitive to inhibition by the FTase inhibitor B956. In contrast, only 50% of K-ras mutant cell lines are inhibited at relatively low (i.e., below 10 µM) concentrations of inhibitor. The remaining 50% of the K-ras-transformed cells tested were inhibited by concentrations of B956 that also inhibit non-ras-mutated cell lines (i.e., between 10 and 100 µM).

![Table 1 Human tumor cell lines](image)

**Fig. 2. Soft agar colony formation by human tumor cell lines.** The indicated cell lines were grown in soft agar as described in "Materials and Methods." At 14–21 days, colonies were stained and counted. Cell lines are grouped according to the mutant form of ras they express. In contrast, control lines (normal) are not shown. Arrows indicate the position of the parent ras-containing cell line.
Therefore, B1086 was used to test whether tumors induced by human
was observed (Fig. 4A). At this time, tumors from control and B956-
(HT-1080 fibrosarcoma; Fig. 2B) or K-ras (HCT 116
colon carcinoma; Fig. 5C) oncogenes. Tumors induced by EJ-1 cells
grew slowly and were very sensitive to treatment with B1086. The
effect of the inhibitor was dose dependent. At the maximum dose of
100 mg/kg, no apparent tumor growth was observed, and 1 mouse of
had no visible tumor at the end of the experiment. Tumors induced
by EJ-1 cells were found to be quite resistant to conventional antitu-
mor drugs (such as cisplatin and doxorubicin; data not shown). How-
ever, the Ftsae inhibitor B1086 at the dose of 100 mg/kg inhibited
tumor growth completely.

The effect of B1086 on HT-1080-induced tumors is not as striking
as that observed against EJ-1 xenografts. However, there is a statisti-
cally significant difference between the tumor growth of treated and
control animals. The tumors in B1086-treated animals grew more
slowly, and the effect was dose dependent (Fig. 5B). At the end of the
experiment, tumors of animals treated with 100 mg/kg were 40% smaller
than those of control animals (Fig. 5B). HT-1080 xenografts
induce cachexia to the animals; therefore, long experiments are not
possible. Animals bearing tumors in the control group, as well as in
the 25 and 50 mg/kg B1086 treatment groups, showed signs of
necrosis and bleeding at the tumor site. However, animals treated with
100 mg/kg B1086 looked healthier than untreated animals.

The human tumor carcinoma cell line HCT116 was the least sen-
sitive of the tumor xenografts tested (Fig. 5C), despite the fact that
when grown in soft agar it was more sensitive to the inhibitor than
were HT1080 cells (Fig. 2). Changes in growth properties in vitro and
in vivo could account for this difference. Treatment with 100 mg/kg
B1086 significantly reduced tumor size by only 20% (P < 0.05).
Reduction of tumor growth rate was observed between days 11 and
17. By the end of the experiment (i.e., 21 days), no significant
difference between the tumor size of treated and control animals was

Inhibition of Tumor Growth Induced by ras-transformed 3T3
Fibroblasts. Nude mice were implanted s.c. with H-ras-transformed
NIH 3T3 fibroblasts and treated systemically by two daily i.p. injec-
tions of 125 or 250 mg/kg B956 for 5 days. Tumors were measured
every 3–4 days until mice were sacrificed at day 14. The results (Fig.
3) clearly show that B956 treatment inhibits tumor growth in a
dose-dependent manner. Tumor growth resumes after a delay of
several days, depending on the dose of B956 used. The rate of tumor
growth after the delay is similar to that of untreated animals. No signs
of toxicity were observed, and all the animals gained weight normally
during the experiment.

Inhibition of ras Processing Associated with Inhibition of Tu-
more Growth. To determine whether the inhibition of tumor growth
was in fact due to inhibition of ras processing, ras localization was
determined after B956 treatment. For this purpose, mice given im-
plantations of ZH1 cells were treated with B956 6 days after implant-
ation, when the tumor was visible. After a 3-day treatment with the
farnesyl transferase inhibitor, a significant reduction of tumor growth
was observed (Fig. 4A). At this time, tumors from control and B956-
treated animals were excised, homogenized, and separated into mem-
brane and cytosol fractions. Fully processed ras localizes to the
plasma membrane, whereas unprocessed ras, because of the lack of
prenyl anchor, is not membrane associated but accumulates in the
cytosol (16). As expected, all the ras protein was detected in the
membrane fraction of tumor cells from control animals (Fig. 4B, Lane
1). In contrast, in the tumors from B956-treated animals, ras with a
slower mobility accumulated in the cytosol (Fig. 4B, Lane 4), and
almost no ras was detected in the membrane fraction (Fig. 4B, Lane
3). To our knowledge, this is the first demonstration that the decrease
in tumor growth induced by a farnesyl transferase inhibitor is corre-
lated with inhibition of ras farnesylation.

Inhibition of Growth of Human Tumor Xenografts. Preliminary
experiments with the methyl ester produg B956, compound B1086,
indicated that the latter was at least twice as potent at inhibiting tumor
growth induced by 3T3 fibroblasts expressing the H-ras oncogene.
Therefore, B1086 was used to test whether tumors induced by human
tumor cell lines expressing oncogenic ras protein were also sensitive
to FTase inhibitors. Nude mice were given implantations s.c. of
human tumor cells expressing H-ras (EJ-1, bladder carcinoma; Fig.
5A), N-ras (HT-1080 fibrosarcoma; Fig. 5B) or K-ras (HCT 116
colon carcinoma; Fig. 5C) oncogenes. Tumors induced by EJ-1 cells

Fig. 4. ras posttranslational processing and inhibition of tumor growth. A, ZH1 cells
were implanted s.c. into nude mice. At day 6, B956 was administered i.p. at 200 mg/kg
for 7 injections, every 12 h. The tumor volume of control (●) or treated (□) animals was
determined at days 6 and 9. Columns, mean; bars, SD. B, at day 9, tumors were removed
and fractionated into membrane (Lanes 1 and 3) and cytosol (Lanes 2 and 4). Protein from
each fraction (50 μg) was separated by gel electrophoresis and identified by Western
immunoblot with F235-1.7.1 antibody. Lanes 1 and 2, tumors from control, untreated
animals; Lanes 3 and 4, tumors from B956-treated animals.
Fig. 5. Effect of B1086 on human tumor xenografts. EJ-1 (A), HT1080 (B), or HCT116 (C) cells were implanted s.c. into nude mice at day 0 as described in "Materials and Methods." At day 1, mice were separated into control (n = 10) and treatment (n = 5) groups, and i.p. administrations at the times indicated by arrows were started. Control animals (●) received vehicle. Treated animals received 25 (●), 50 (▲), or 100 (○) mg/kg B1086 in saline containing 2% Tween 80. Tumor volume was measured every 3—4 days as shown. Points, tumor volume (mm³); bars, SD.

observed (Fig. 5C). The size of tumors in untreated animals reached saturation at 17 days, probably because of the cachexia induced in the animals by these tumor cells. However, as observed above for HT1080 xenografts, the cachetic effect of HCT116 cells was significantly decreased in animals treated with 100 mg/kg B1086.

DISCUSSION

The number of reports on new synthetic inhibitors of FTase activity in vitro is growing (Refs. 14, 15, and 17–20 contain just a few examples of inhibitors). However, there are few reports demonstrating in vivo efficacy (21, 22). In the present report, it is shown that the peptidomimetic B956 and its methyl ester prodrug B1086 are potent not only against model cell lines of ras-transformed cells but also against tumor cell lines of human origin that express different ras oncogenes. In our in vivo model system, the potency of B1086 was comparable to that of L739,749 (Ref. 21; data not shown). It is also shown that inhibition of tumor growth by this FTase inhibitor is associated with inhibition of ras posttranslational processing in the tumor (Fig. 4). As it has been indicated by others (21), the use of FTase inhibitors appears to be safe and has potential for use as long-term therapy: after 18 daily administrations of B1086, no signs of toxicity were evident.

Whereas the ester prodrug was about 10 times more potent than the acid in intact cells, presumably because of a higher membrane permeability, there was only a 2-fold increase in potency in vivo. Preliminary pharmacokinetic data indicates that the ester prodrug is not very stable in circulation because it is hydrolyzed to the acid within 5 min of administration. Thus, the ester probably increases the prodrug permeability across the epithelial barrier, but its rapid hydrolysis results in a higher circulating concentration of the active but less permeable form, B956. It was also found that the inhibitor accumulates in the tumor at similar levels as in plasma, but its clearance from the tumor is slower.

The effect of B1086 or B956 on tumor growth was transient. Once the drug was removed, the tumors resumed growth at approximately the same rate as untreated controls, indicating a cytostatic and not a cytotoxic effect. However, the results with EJ-1 xenografts (Fig. 5A) suggest that with a more optimized treatment strategy, the inhibition of tumor growth could be irreversible and complete. In this case, a longer treatment schedule was devised to accomodate the slow growth rate of EJ-1 tumors. At the end of the experiment, one mouse in the higher dose group was tumor free, whereas the remaining 4 animals did not show apparent tumor growth. Although it is possible that tumors would grow during a longer observation period, this result suggests that an adequate treatment schedule might eliminate tumor growth completely. Further support for this idea comes from the experiment where treatment of animals with a growing tumor resulted in a significant reduction of tumor growth (Fig. 4A). In most of our experiments, drug treatment is started soon after tumor implantation because under this condition tumors are more sensitive. However, this experiment shows that the growth of an already present tumor can be slowed down and possibly stopped by FTase inhibitors using an appropriate dosage regimen.

One piece of puzzling information is derived from data with a panel of human tumor cell lines expressing different ras oncogenes. When these cell lines are treated with B956 in a colony-forming assay, differences in sensitivities are observed. We have shown previously that increasing concentrations of inhibitor are required to inhibit posttranslational processing of different prenylated proteins (18). However, in this case, differences in sensitivities are observed among cell lines expressing the same ras oncogene. This was especially clear for cells expressing the K-ras oncogene, where a large number of cell lines was tested. It is possible that a wide range in sensitivity to FTase inhibitors will be observed in cells with H- or N-ras mutations if more cell lines are studied. On the other hand, whereas other reports have shown lack of effect of FTase inhibitors on non-ras-transformed cell lines (i.e., raf-, mos-, or src-transformed cell lines; Refs. 14 and 17), the data presented here indicate that about 50% of the K-ras-transformed cell lines tested are as sensitive as non-ras-transformed cell lines. This discrepancy could perhaps be eliminated if more cell lines are studied in different laboratories.

Available data indicate that if a cell line expresses a ras oncogene, its growth is governed by the presence of activated ras (23). However,
tumor cell lines are characterized by the presence of multiple mutations in different genes. It is possible that unidentified mutations downstream of ras could maintain cell growth, even if ras signaling is disrupted by FTase inhibitors. Alternatively, mutations in pathways parallel to ras could be sufficient to maintain the transformed phenotype. The above alternatives might explain why a ras-transformed cell line could be resistant to growth inhibition by farnesylation inhibitors. The opposite (namely, activation of factors upstream of ras) could explain why cells without mutant forms of ras are sensitive to these inhibitors. For instance, an activated growth factor receptor or unidentified oncogenes upstream of ras could activate ras normally and continuously. One example of the latter is found in chronic myelogenous leukemias, where bcr/abl is presumed to activate ras by phosphorylation of the grb2 adaptor protein (24–26). In such cases, cell growth could be further reduced by inhibition of ras farnesylation. These hypotheses deserve further testing. They suggest that, at the clinical level, it might be necessary to test for ras dependency of tumor growth before deciding on a treatment strategy. Whereas this might reduce the number of treatable tumors where the ras oncogene is present, it will add other tumors that are not obvious targets at the moment.

In summary, the data presented here confirm the hypothesis that, for ras-dependent tumors, inhibition of ras farnesylation results in inhibition of ras function and, therefore, inhibition of tumor growth. It seems plausible that a tumor might be ras dependent, even in the absence of the ras oncogene, thus broadening the spectrum of tumor targets for FTase inhibitors.

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