Both Farnesylated and Geranylgeranylated RhoB Inhibit Malignant Transformation and Suppress Human Tumor Growth in Nude Mice*

Zhi Chen‡, Jiazh Sun‡, Anne Pradines§, Gilles Favre§, Jalila Adnan‡, and Said M. Sebit‡

From the ‡Drug Discovery Program, H. Lee Moffitt Cancer Center and Research Institute, Department of Biochemistry and Molecular Biology, University of South Florida, Tampa, Florida 33612 and §Institut de Biologie et de Biotechnologie, UPRES EA 2048, Université Paul Sabatier and Centre de Lutte Contre le Cancer Claudius Regaud, Toulouse 31052, France

Received for publication, March 3, 2000, and in revised form, April 14, 2000
Published, JBC Papers in Press, April 17, 2000, DOI 10.1074/jbc.C000145200

Whereas the GTPase RhoA has been shown to promote proliferation and malignant transformation, the involvement of RhoB in these processes is not well understood. In this manuscript RhoB is shown to be a potent suppressor of transformation and human tumor growth in nude mice. In several human cancer cell lines, RhoA promotes focus formation whereas RhoB is as potent as the tumor suppressor p53 at inhibiting transformation in this assay. RhoB is both farnesylated (F) and geranylgeranylated (GG), and RhoB-F has been suggested as a target for the antitumor activity of farnesyltransferase inhibitors. Here we demonstrate that both RhoB-F and RhoB-GG inhibit anchorage-dependent and -independent growth, induce apoptosis, inhibit constitutive activation of Erk and insulin-like growth factor-1 stimulation of Akt, and suppress tumor growth in nude mice. The data demonstrate that RhoB is a potent suppressor of human tumor growth and that RhoB-F is not a target for farnesyltransferase inhibitors.

Low molecular weight GTP/GDP-binding GTPases such as Ras and Rho transduce mitogenic and survival signals from cell surface receptor to the nucleus (1–8). For example, platelet-derived growth factor and insulin-like growth factor-1 (IGF-1) stimulate cell proliferation and survival by activating their receptor tyrosine kinases, which recruit nucleotide exchange factors that activate Ras by converting it to its GTP-bound state. Once activated, Ras triggers a complex set of signal transduction pathways. These include the phosphatidylinositol 3-kinase/Akt pathway believed to be critical for cell survival and the Raf/Mek/Erk kinase cascade that has been implicated in cell proliferation (1–3). In addition to its involvement in regulating proliferation and survival, Ras also plays a pivotal role in malignant transformation. In about 30% of all human cancers, Ras is found mutated to a GTPase-deficient form that leads to constitutive activation of the above signaling pathways, uncontrolled proliferation, and survival of human tumors (4, 5).

Closely related family members to Ras, such as RhoA and Rac1, have also been shown to be intimately involved in proliferation and transformation (3). For example both RhoA and Rac1 are required for the G1 to S phase transition during the cell division cycle (6). Furthermore, GTP-locked RhoA and Rac1 are transforming, and dominant negative forms of these GTPases inhibit Ras-induced malignant transformation (7, 8). Unlike RhoA and Rac1, less is known about the involvement of the RhoB GTPase in proliferation and transformation. There are several features that distinguish RhoB from other Rho proteins. First, its cellular localization in early endosomes and prelysosomal compartment is different from other members (9). Moreover, RhoB is an immediate early response gene that is induced by platelet-derived growth factor, transforming growth factor-α, the non-receptor tyrosine kinase v-Src, and ultraviolet irradiation (3, 10, 11). However, these studies were performed in fibroblasts mainly, and whether RhoB is also an immediate early response gene in human cancer cells of epithelial origin is not known. Finally, RhoB mRNA and protein levels are turned over much more rapidly (half-lives of 20 and 120 min, respectively) than other GTPases, which typically have half-lives on the order of 24 h (2, 3). Therefore, although RhoA and RhoB share 90% amino acid sequence homology, their physiological functions are predicted to be distinct.

Low molecular weight GTPases require prenylation, a lipid post-translational modification, for their biological activity (12). The two enzymes that catalyze these modifications for Ras and Rho GTPases are farnesyltransferase (FTase) and geranylgeranyltransferase I (GGTase I). The enzymes recognize the C-terminal sequence CAXX (C = cysteine, A = aliphatic amino acid, and X = any amino acid) and covalently attach a farnesyl or a geranylgeranyl to the cysteine. FTase prefers a methionine or a serine whereas GGTase I prefers a leucine at the X position. Ras proteins (e.g. Ha-, Ki-, and N-Ras) are farnesylated, whereas RhoA and Rac1 are geranylgeranylated (12). Although RhoB has a C-terminal leucine, which would be predicted to dictate only geranylgeranylation, it is both farnesylated and geranylgeranylated in cells (12). Because RhoB is found constitutively activated in 30% of human cancers (4, 5) and Ras farnesylation is required for its malignant transforming activity (13), FTase inhibitors (FTIs) were designed as novel anticancer drugs (14–16). FTIs have shown impressive antitumor activity and lack of toxicity in preclinical models and are presently being used in various human clinical trial phases (14–16). Although initially FTIs were hypothesized to inhibit tumor growth by targeting Ras, recent evidence suggests that other...
RhoB inhibits human tumor growth in nude mice

farnesylated proteins may be involved (17). RhoB has been suggested as a potential candidate for several reasons. First it is a substrate for FTase, and FTIs inhibit its farnesylation resulting in decreased RhoB-F and increased RhoB-GG. Second, the short half-life of RhoB coincides better than that of Ras with regard to the kinetics of the reversal of transformation of FTI (17). Third, a RhoB/RhoA chimeric protein that is exclusively geranylgeranylated was shown to be growth-inhibitory. Finally, a myristylated form of RhoB that is not prenylated was shown to prevent the ability of FTIs to inhibit Ras transformation (17). However, the biochemical properties of myristylated RhoB are not the same as wild type RhoB making it difficult to interpret the data. Furthermore, RhoB has been shown to be farnesylated by FTase as well as by GTase I. Finally, most of the studies carried out so far were in murine fibroblasts (17). Therefore, although there is some evidence suggesting the involvement of RhoB in the antitumor activity of FTIs, direct evidence implicating RhoB in the mechanism of action of FTIs in human tumors is lacking. One way to directly address this important issue is to design RhoB mutants with CAAX boxes that are either exclusively farnesylated or geranylgeranylated and to determine whether RhoB-F is transforming and RhoB-GG is anti-transforming in human cancer cells. In this manuscript we describe a novel function for RhoB(WT) as a potent inhibitor of malignant transformation and a suppressor of human tumor growth. Furthermore, both RhoB-F and RhoB-GG induce apoptosis, inhibit oncogenic signaling, and suppress transformation in vitro and in vivo. These findings demonstrate the tumor-suppressing activity of RhoB and strongly suggest that RhoB-F is not a target for FTIs in human cancer cells.

EXPERIMENTAL PROCEDURES

Constructs—Wild type RhoB-WT, which has a CKVL sequence for a CAAX box and which is both farnesylated and geranylgeranylated, was used to make the following CAAX box mutants: RhoB-CAIM (F) designed to be only farnesylated and RhoB-CLLL (GG) designed to be only geranylgeranylated. All mutants were sequenced and found to have correct mutations. Also, all mutants were shown to have the correct prenylation status by transfecting each construct into COS7 cells, immunoprecipitating RhoB, cleaving the prenyl group, and analyzing the nature of the prenyl by high performance liquid chromatography.2 Wild type RhoA was obtained from Dr. Channing Der (University of North Carolina, Chapel Hill, NC) and subcloned into pcDNA3. Focus Formation Assay—Panc-1 and Saos-2 cell lines (ATCC) were derived from human pancreatic and osteosarcoma, respectively. HeLa and C-33A (ATCC) were derived from human cervical carcinoma. Panc-1, Saos-2, C-33A, and HeLa were maintained in DMEM supplemented with 10% FBS. One day prior to transfection, 2×106 cells were seeded into 60-mm plates. Cells were transfected with 1 μg of each expression vector using Fugene6 (Roche Molecular Biochemicals) following the manufacturer’s recommendation. Two days post-transfection cells were collected with trypsin, counted, and seeded into 100-mm plates at a density of 5×104 per plate. RhoA and p53 are expressed from pcDNA3neo vector (Invitrogen) whereas RhoB-wt, RhoB-F, and RhoB-GG were expressed from pCMV-IRES/Zeo vector.2 Zeocin (Invitrogen) was used as a selection drug for cells transfected with RhoB expression vectors, and G418 (Mediatech, Inc.) was used for cells transfected with RhoA and p53. Zeocin was used at a concentration of 300 μg/ml for Panc-1 and 150 μg/ml for HeLa, Saos-2, and C-33A, whereas G418 was used at a concentration of 800 μg/ml for Panc-1 and 400 μg/ml for HeLa, Saos-2, and C-33A. Cells were cultured in the presence of selection drug for 2 weeks before being fixed and stained with KaryoMax Giemsa stain (Life Technologies, Inc.). Briefly, the medium was discarded, and cells were washed once with PBS and once with PBS/ methanol (1:1). 50% of the methanol/PBS mixture was replaced with fresh methanol and left for 10 min. The mixture was discarded, and cells were washed with fresh anhydrous methanol. The monolayer was then covered with Giemsa stain for 2 min. Finally, the stain was displaced with water.

Generation of Stably Transfected Panc-1 Cells—Panc-1 cells were grown in DMEM, 10% FBS. Different RhoB constructs (RhoB-F, RhoB-GG, or RhoB-WT) and the corresponding empty vector were transfected into Panc-1 cells by using the calcium phosphate method. At day 0, Panc-1 cells were plated (1.7×105 cells/plate), RhoB mutant constructs were transfected (day 1), and growth medium was changed (day 2). Cells were split (day 3), and fresh growth medium containing selection marker, Zeocin (300 μg/ml), was changed every 3–4 days, until colonies formed 2 weeks later. Stably transfected cell lines were expanded and frozen in liquid nitrogen for future use. Soft agar assay was carried out at described previously (18).

Anchorage-dependent Growth—Stably transfected Panc-1 cells expressing the different RhoB mutants were plated in 10% FBS. The number of cells in each dish was counted on days 1, 4, 5, 6, and 7. Cells were counted by hemacytometer.

Apoptotic Assay—To analyze cells undergoing apoptosis, the terminal deoxynucleotidyltransferase dioxogenin nick end-labeling with the ApopTag Fluorescein In Situ Apoptosis detection kit was used. Cells were trypsinized, washed with PBS, and fixed in 1% paraformaldehyde in PBS. After cytoxin and washes with PBS, terminal deoxynucleotidyltransferase enzyme was applied to the cells followed by anti-digoxigenin-fluorescein. Mounting medium containing 4,6-diamidino-2-phenylindole was used to counterstain the nuclei. The cells were viewed by fluorescence microscopy.

Western Blot Analysis—Panc-1 cells stably expressing different RhoB constructs were grown in DMEM, 0.5% FBS for 48 h, treated with or without IGF-1 (50 ng/ml), and processed for Western blotting as described previously (18). Phospho-Akt was analyzed by using anti-phospho-Akt antibody (New England Biolabs). Phospho-Erk was detected by anti-phospho-ERK1/ERK2 antibody (New England Biolabs). Nonphosphorylated Akt and Erk2 were detected by anti-Akt (Santa Cruz Biotechnology) and anti-Erk2 (Upstate Biotechnology). RhoB protein was detected by mouse monoclonal anti-RhoB antibody (Santa Cruz). For phospho-Akt and phospho-Erk2, bands were quantified by using a scanning densitometer Model IGS-700 (Bio-Rad).

Mouse Tumor Xenograft Model—Nude mice (Harlan Sprague-Dawley, Indianapolis, IN) were maintained in accordance with the Institutional Animal Care and Use Committee (IACUC) procedures and guidelines. Panc-1 cells stably expressing RhoB-WT, RhoB-GG, RhoB-F, and empty vector were harvested, resuspended in PBS, and implanted subcutaneously into the right and left flank (10×105 cells/flank) of 8-week-old female nude mice, and the tumors were measured as described previously (18). Statistical significance between empty vector and different stably transfected RhoB mutants was evaluated by using Student’s t test (p<0.05).

RESULTS AND DISCUSSION

Rho B-F, RhoB-GG, RhoB-WT, and p53, but Not RhoA, Suppress Focus Formation of Several Human Cancer Cell Lines—To test the hypothesis that RhoB-F is transforming and that RhoB-GG is anti-transforming, we have generated CAAX box mutants of RhoB that are either exclusively farnesylated or geranylgeranylated. The biochemical demonstration that in whole cells RhoB-F and RhoB-GG are exclusively farnesylated and geranylgeranylated, respectively, and RhoB-WT is farnesylated and geranylgeranylated was provided elsewhere.2 To determine the effects of the RhoB mutants on transformation of human cancer cells we used several in vitro and in vivo assays. We first determined the effects of the RhoB mutants on the ability of human cancer cells to grow foci in a focus formation assay. We transfected several human cancer cell lines with the RhoB mutant DNAs, and the foci that formed were scored 14 days later as described under “Experimental Procedures.” Control transfections were also performed with the tetracycline suppressor p53 and the GTPase RhoA, a closely related RhoB family member that shares 90% amino acid homology and that was previously shown to be transforming (3, 7, 8). Fig. 1A and Table I show that the human pancreatic cancer cell line, Panc-1, transfected with empty vector DNA, pCMV, formed numerous foci (155–233 foci). In contrast, Panc-1 cells transfected with wild-type RhoB grew only 23–39 foci. Furthermore, RhoA-transfected Panc-1 cells grew more foci (over 346–409) than

2 R. Baron, E. Fourcade, L. Lajoie-Mazenc, C. Allal, B. Couderc, R. Barbaras, G. Favre, J.-C. Fay, and A. Pradies, submitted for publication.
RhoB Inhibits Human Tumor Growth in Nude Mice

Both RhoB-F and RhoB-GG as Well as RhoB-WT Inhibit Anchorage-independent and -dependent Growth of Panc-1 Cells—To confirm the effects of the RhoB mutants on malignant transformation, we transfected Panc-1 cells with the above constructs and isolated several stable clones as described under “Experimental Procedures.” Expression of RhoB was controlled by Western blotting using several clones picked from each construction (Fig. 1B). Representative clones with expression levels similar to each other were selected for further studies. The clones picked for further study were RhoB-WT clone 2 (W2), RhoB-F clone 2 (F2), and Rho-GG clone 2 (G2) (Fig. 1B). We next determined the effects of the different RhoB mutants on the ability of Panc-1 cells to grow on soft agar. Panc-1 cells stably transfected with either empty vector, RhoB-F, RhoB-GG, or RhoB-WT were plated on soft agar plates and developed 3 weeks later as described under “Experimental Procedures.” Fig. 1C shows that Panc-1 cells transfected with empty vector grew numerous and large colonies. In contrast, Panc-1 cells expressing RhoB-WT, RhoB-F, and RhoB-GG show little growth on soft agar. RhoB wild type was very potent at inhibiting Panc-1 soft agar growth, and no colonies could be detected (Fig. 1C). Thus, the results of Fig. 1C are in agreement with those of Fig. 1A and Table I and demonstrate that RhoB antagonizes tumor growth and suggest that RhoB-F is not a target for the antitumor activity of FTIs in human cancer cells.

Anchorage-dependent and -independent growth in Panc-1 cells could be regulated by different mechanisms. We therefore determined the effect of the RhoB mutants on the anchorage-dependent growth of Panc-1 cells. The different Panc-1 cell lines were plated on plastic dishes, and the growth rate of each cell line was determined by counting cells for 7 days as described under “Experimental Procedures.” Fig. 2A shows that empty vector Panc-1 cells grew the fastest and reached 1.34 x 10^6 cells over 7 days. In contrast, RhoB-WT cells grew the least and reached only 0.43 x 10^6 cells over the same period of time. RhoB-F and RhoB-GG also grew slower reaching 0.64 x 10^6 and 0.93 x 10^6 cells over 7 days. Thus, RhoB inhibits both anchorage-dependent and -independent growth of Panc-1 cells, but the effect on anchorage-independent growth was more pronounced for all mutants.

RhoB-F, RhoB-GG, and RhoB-WT Induce Apoptosis with Little Effect on Cell Cycle Distribution—The ability of RhoB mutants to inhibit anchorage-dependent and -independent growth could be due to cell cycle arrest and/or apoptosis. We determined the ability of RhoB mutants to alter cell cycle distribution by flow cytometry and found that RhoB mutants have little effect on cell cycle distribution (data not shown). We next evaluated whether or not the RhoB mutants affect programmed cell death of Panc-1 cells using ApopTag DNA fragmentation assays as described under “Experimental Procedures.” Fig. 2B shows that only 7% Panc-1 cells transfected with empty vector were undergoing apoptosis. In contrast, 21, 14, and 13% of RhoB-WT, RhoB-F, and Rho-GG cells, respectively, were in apoptosis.

Table I: anchorage-independent and -dependent growth of Panc-1 cells—

| Human cancer cell line | pCMV | RhoB-WT | RhoB-GG | RhoB-F | pCDNA3 | RhoA | p53 |
|------------------------|------|--------|--------|-------|--------|-----|-----|
| Panc-1                 | 155  | 23     | 27     | 30    | 163    | 346 | 21  |
| Panc-1                 | 233  | 39     | 43     | 49    | 211    | 409 | 27  |
| HeLa                   | 26   | 1      | 0      | 2     | 31     | 46  | 0   |
| HeLa                   | 57   | 4      | 3      | 7     | 65     | 89  | 2   |
| C-33A                  | 73   | 2      | 17     | 23    | 191    | 263 | 14  |
| C-33A                  | 61   | 5      | 9      | 16    | 221    | 287 | 21  |
| Saos-2                 | 26   | 0      | 0      | 2     | 46     | 61  | 1   |
RhoB inhibits human tumor growth in nude mice

A

Fig. 2. RhoB inhibits anchorage-dependent growth and induces apoptosis in Panc-1 cells. A, Panc-1 cells stably transfected either with empty vector (•), RhoB-GG (▲), RhoB-F (■), or RhoB-WT (◆) were plated in 60-mm plates (1.7 × 10⁴ cells/plate). The cells were then harvested at days 1, 4, 5, and 7 and counted. Data are representative of three independent experiments. B, Panc-1 cells stably expressing RhoB-WT, RhoB-F, RhoB-GG, or empty vector (pCMV) were plated in 10% FBS on day 1, harvested on day 3, and processed for ApopTag apoptosis assay as described in “Experimental Procedures.” The number of cells undergoing apoptosis was counted. About 500 cells were counted for each cell line. The percentage of apoptotic cells was determined as the percentage of bright green cells among the total number of cells. Data represent the average numbers and standard error of three independent experiments. * designates p < 0.05 between pCMV and the various mutants.

RhoB-F, RhoB-GG, and RhoB-WT Inhibit IGF-1 Stimulation of Akt and Constitutive Activation of Erk1/2—A possible mechanism by which RhoB would enhance the ability of Panc-1 cells to undergo apoptosis is by inhibiting a survival pathway. One of the major signal transduction pathways that contributes to cell survival and prevention of cell death is the growth factor-stimulated phosphatidylinositol 3-kinase/Akt pathway where the Ser/Thr kinase Akt plays a pivotal role (2). We, therefore, determined the effects of the various RhoB mutants on the ability of one of the major survival growth factors, IGF-1, to stimulate Akt by Western immunoblotting with an antibody specific for activated (phosphorylated) Akt as described under “Experimental Procedures.” Fig. 3 shows that IGF-1 treatment of serum-starved Panc-1 cells transfected with empty vector resulted in potent activation of Akt. In contrast, in Panc-1 cells overexpressing RhoB-F, RhoB-GG, and RhoB-WT, the ability of IGF-1 to stimulate Akt phosphorylation/activation was inhibited by 50% (± 10%), 50% (± 18%), and 65% (± 15%), respectively. None of the RhoB mutants affected the expression levels of Akt (Fig. 3).

We next evaluated the effects of the RhoB mutants on the activation of Erk1 and Erk2, two mitogen-activated protein kinases that have been shown to play a pivotal role in transformation (2, 3). These experiments were carried out in parallel with the experiments described above for Akt except that activation of Erk1/Erk2 was determined by immunoblotting using an antibody specific for activated (phosphorylated) Erk1/Erk2 as described under “Experimental Procedures.” Unlike Akt, treatment with IGF-1 did not further stimulate Erk1 and Erk2 (Fig. 3). Furthermore, in Panc-1 cells Erk2, but not Erk1, is constitutively activated as is apparent from the strong hyperphosphorylated band. Fig. 3 shows that RhoB-WT, RhoB-GG, and RhoB-F inhibited Erk2 constitutive activation by 75%, 70%, and 60%, respectively. None of the RhoB mutants affected the expression levels of Erk2 (Fig. 3).

RhoB-F, RhoB-GG, and RhoB-WT Suppress the Growth of Panc-1 Tumor Cells in Nude Mice—The results described above demonstrate that RhoB inhibits proliferation, foci formation, and soft agar growth, induces apoptosis, and inhibits signal transduction pathways involved in survival and transformation. The data indicate that RhoB is a potent suppressor of malignant transformation in Panc-1 cells in vitro. We next evaluated the ability of the RhoB mutants to suppress malignant transformation in an in vivo environment by evaluating their effects on the growth of Panc-1 cells in nude mice. Panc-1 cells expressing the different RhoB mutants (10⁵ cells/flank) were implanted subcutaneously under the right and left flank of nude mice. The tumor growth of the different Panc-1 cells was then followed by caliper measurements of the tumor sizes over time as described under “Experimental Procedures.” Fig. 4 shows that within 5 days of cell implantation under the skin of nude mice, empty vector Panc-1 cells had an average tumor size of 116 ± 10. In contrast, cells stably expressing RhoB-GG, RhoB-WT, or RhoB-F were smaller and flatter in appearance and had an average tumor size of 67 ± 6, 91 ± 9, and 95 ± 8 mm³, respectively. Fig. 4 also shows that empty vector cells grew whereas RhoB-WT-, RhoB-F-, and RhoB-GG-expressing Panc-1 cells regressed. Tumor regression was the fastest with
Panc-1 cells stably expressing RhoB-WT, and within 15 days of tumor implantation, all tumors disappeared. Panc-1 cells stably expressing RhoB-F and RhoB-GG took longer to disappear (43 and 56 days, respectively). Over a 75-day period since tumor cell implantation, empty vector cells grew to an average size of 455 ± 197 whereas RhoB-F, RhoB-GG, and RhoB-WT remained undetectable (Fig. 4). Thus, RhoB-F, RhoB-GG, and RhoB-WT are all potent suppressors of Panc-1 tumor growth in nude mice. The ability of RhoB to suppress Panc-1 cell tumor growth in nude mice requires prenylation by either farnesyl or geranylgeranyl because a RhoB mutant that lacks a CAAX box did not inhibit tumor growth (Fig. 4).

In this manuscript we provided strong evidence for a growth-inhibitory and tumor suppressor activity of the small GTPase RhoB. Little is known about the role of RhoB in proliferation and transformation of human cancer cells. In Rat1 fibroblasts, a dominant negative form of RhoB was shown to weakly inhibit focus formation induced by oncoenic Ras (19). However, activated (GTPase-deficient Val-14-RhoB) itself lacked focus formation activity arguing against a transforming role for RhoB (19). In the present study, we demonstrated in human cancer cells that RhoB was a potent inhibitor of tumor growth of these cells in nude mice. Furthermore, RhoB was also a potent inhibitor of human tumor growth in vitro as shown in both anchorage-dependent (proliferation) and -independent (transformation) assays in vitro. RhoB is as potent as p53 in suppressing foci formation in several human cancer cell lines. In the same in vitro transformation assay RhoA enhances foci formation. The ability of RhoB to inhibit focus formation is not dependent on the Ras mutation status of the human cancer cell lines. Indeed, RhoB was a potent inhibitor in cancer cells where Ras is mutated (Panc-1) as well as those where Ras is wild type (HeLa, C-33A, and Saos-2). The tumor growth suppressor activity of RhoB was also p53-independent because some of the human cancer cell lines used have nonfunctional p53. Furthermore, RhoB disrupted two major signaling pathways by inhibiting IGF-1 stimulation of Akt and constitutive activation of Erk2. This is consistent with its ability to inhibit proliferation and transformation. Thus, the ability of RhoB to block two signaling pathways that are involved in tumor survival and transformation may be pivotal to the tumor-suppressive activity of Panc-1 cells in nude mice.

The ability of RhoB to potently antagonize transformation in vitro and in vivo suggested that it is most likely not a target for the antitumor activity of FTI in human cancer cells. However, considering that RhoB is both farnesylated and geranylgeranylated in cells (12, 13), it is conceivable that the antitransforming activity of RhoB is mainly due to its geranylgeranylated form and that in its farnesylated state RhoB is transforming. Indeed it was recently suggested that RhoB-F induces tumor survival and that RhoB-GG inhibits tumor growth (17). It was further suggested that FTI treatment would result in loss of the survival function of RhoB-F (apoptosis/cytotoxic effects) and gain of the growth-inhibitory function of RhoB-GG (cell cycle/cytostatic effects) (17). However, this hypothesis is based on evidence derived from results obtained mainly from studies undertaken in Ras-transformed fibroblasts. Although in fibroblasts this may be the case, our results argue against this hypothesis in human cancer cells. RhoB-F was just as potent as RhoB-GG at inhibiting human tumor growth in vitro and IGF-1 stimulation of Akt and constitutive activation of Erk2 as well as inducing apoptosis and inhibiting tumor growth in nude mice. Thus, the work described in this manuscript identifies the potent antitransforming activity and tumor suppressor activity of RhoB and provides strong evidence that in human cancer cells, inhibition of the farnesylation of RhoB does not contribute to the mechanism by which FTIs inhibit tumor growth.

Acknowledgments—We thank the Moffitt Cancer Center flow cytometry core facility for their help. We also thank Dr. Alan Cantor (Program Leader, Biostatistics Program) for assistance with statistical analysis.

REFERENCES

1. McCormick, F. (1993) Nature 363, 15–16
2. Campbell, S. L., Khorosavi-Far, R., Rossman, K. L., Clark, G. J., and Der, C. J. (1998) Oncogene 17, 1395–1413
3. Zohn, I. M., Campbell, S. L., Khorosavi-Far, R., Rossman, K. L., and Der, C. J. (1998) Oncogene 17, 1415–1438
4. Barbacid, M. (1987) Annu. Rev. Biochem. 56, 779–827
5. Bos, J. L. (1989) Cancer Res. 49, 4682–4689
6. Olsen, M. F., Ashworth, A., and Hall, A. (1995) Science 269, 1270–1272
7. Khorosavi-Far, R., Solski, P. A., Clark, G. J., Kinch, M. S., and Der, C. J. (1995) Mol. Cell. Biol. 15, 6443–6453
8. Qu, R. G., Chen, J., Kirn, D., McCormick, F., and Symons, M. (1995) Nature 374, 457–459
9. Mellor, H., Flynn, P., Nokes, C. D., Hall, A., and Parker, P. J. (1998) J. Biol. Chem. 273, 4811–4814
10. Jahner, D., and Hunter, T. (1991) Mol. Cell. Biol. 11, 3682–3690
11. Fritz, G., Kainz, B., and Aktories, K. (1995) J. Biol. Chem. 270, 25172–25177
12. Zhang, P. L., and Casey, P. J. (1996) Annu. Rev. Biochem. 65, 241–269
13. Lebowitz, P. F., Casey, P. J., Prendergast, G. C., and Thissen, J. A. (1997) J. Biol. Chem. 272, 15591–15594
14. Sebti, S. M., and Hamilton, A. D. (1997) Pharmaceut. Ther. 74, 103–114
15. Gibbs, J. B., and Off, A. (1997) Annu. Rev. Pharmacol. Toxicol. 37, 143–166
16. Cox, A. D., and Der, C. J. (1997) Biochim. Biophys. Acta 1333, F51–F71
17. Lebowitz, P. F., and Prendergast, G. C. (1998) Oncogene 17, 1439–1445
18. Sun, J., Blaskovich, M. A., Knowles, D., Qian, Y., Ohkanda, J., Bailey, R. D., Hamilton, A. D., and Sebti, S. M. (1999) Cancer Res. 59, 4919–4926
19. Prendergast, G. C., Khorosavi-Far, R., Solski, P. A., Kurzawa, H., Lebowitz, P. F., and Der, C. J. (1995) Oncogene 10, 2269–2276
