Palmitoylated Cysteine 192 Is Required for RhoB Tumor-suppressive and Apoptotic Activities*  

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RhoA and RhoB share 86% amino acid sequence identity, yet RhoA promotes whereas RhoB suppresses malignant transformation. Amino acids 29, 100, 116, 123, 129, 140–143, 141, 146, 152, 154, 155, 173, 181, 185–187, 189, 190, 191, 192, and 193 in RhoB were mutated to the corresponding RhoA residues to determine those critical for RhoB tumor-suppressive activity. Of all the mutants made, only the cysteine 192 (one of two palmitoylation sites) and cysteine 193 (the prenylation site) point mutations abolish RhoB functions. In contrast, mutation of the other palmitoylation site, cysteine 189, did not affect RhoB functions. Moving cysteine 192 to position 190 did not affect RhoB function either. Mutation of cysteine 192 to glycine, alanine, or serine blocks the ability of RhoB to suppress transforming growth factor β type II receptor, p21<sup>raf</sup>, and AP-1 promoter transcriptional activities. Furthermore, mutations of cysteines 192 and 193, but not 189, mislocalize RhoB and prevent RhoB from inhibiting anchorage-dependent and anchorage-independent tumor growth and colony formation as well as prevent it from inducing apoptosis. The cysteine 192 RhoB mutant is far less active and geranylgeranylated as efficiently as wild type RhoB. A RhoA-(1–180)/RhoB-(181–196) chimera inhibited tumor cell proliferation and induced apoptosis as efficiently as RhoB. These results demonstrate that the presence of neither cysteine 193 nor cysteine 192 alone is sufficient and that both palmitoylated cysteine 192 and prenylated cysteine 193, but not palmitoylated cysteine 189, are required for RhoB tumor-suppressive and pro-apoptotic activities.

Rho proteins are GTP/GDP binding GTPases that belong to the Ras superfamily and are intimately involved in diverse cellular processes and diseases (1). For example, Rho proteins are pivotal in the regulation of actin cytoskeleton processes such as lamellipodia, fiber, and membrane ruffle formation (2, 3). Rho proteins also regulate signal transduction proteins such as extracellular signal-regulated kinase (ERK), p38, and stress-activated protein kinase (SAPK) that are involved in the mitogen- and stress-activated kinase pathways (4). Most important is the involvement of Rho GTPases as mediators of proliferation and malignant transformation. For example, RhoA and Rac1 are critical for the G<sub>1</sub>/S cell division cycle traverse (5) and mediate oncopgenic Ras malignant transformation (5, 6). In cellular and animal models Rho proteins such as RhoA, Rac1, Cdc42, and RhoC have been implicated in invasion and metastasis, and RhoC has been shown to contribute to metastasis in clinical settings (5–7).

Although most Rho proteins are involved in promoting oncogenesis, invasion, and/or metastasis, mounting evidence points to a tumor-suppressive role for RhoB. First, in cultured cells RhoB inhibits oncogenic signaling (8, 9) as well as anchorage-dependent and -independent tumor cell growth (8) and induces apoptosis (8, 10). Second, ectopic expression of RhoB suppresses the growth of human cancer cells in nude mice (8, 11). Third, RhoB knockout mice are more sensitive to chemically induced tumors (12), and RhoB (−/−) cells are resistant to apoptosis induced by radiation and cytotoxic agents (13). Fourth, ectopic expression of RhoB suppresses epidermal growth factor receptor-, ErbB2-, Ras-, phosphatidylinositol 3-kinase-, and Akt-induced tumor survival, proliferation, invasion, and metastasis (11, 14). Fifth, many oncopgenes such as the epidermal growth factor receptor, Ras, and Akt suppress the expression of RhoB (11, 14). Finally, in patients with head and neck, lung, and brain cancers, RhoB protein levels are drastically decreased as the tumors become more aggressive and highly invasive (15–17). The above studies suggest that RhoB plays a critical role in suppressing malignant transformation by blocking oncogenic and tumor survival pathways and that oncopgenes such as Ras and the epidermal growth factor receptor suppress RhoB expression as a step toward malignant transformation.

The fact that RhoA and RhoB have opposing effects on malignant transformation is intriguing, considering that RhoA and RhoB share 86% amino acid identity. Presently, it is not understood why RhoA promotes, whereas RhoB suppresses, malignant transformation. In this manuscript we have carried out site-directed mutagenesis studies with the goal of identifying those amino acids in RhoB that are critical to its tumor-suppressive activity.

**EXPERIMENTAL PROCEDURES**

**Site-directed Mutagenesis—**Site-specific mutations were generated using the ExSite™ mutagenesis kit (Stratagene). Target genes were modified by introducing a silent mutation to provide a new restriction site in the construct without affecting the coding residue (18). For the chimera construct, the boldfaced sequences shown in Table I are from RhoB, and the rest of the primer sequence was matched with RhoA (Table I). All mutants were verified by complete sequencing. The corresponding primers and silent mutation of all mutants are shown in the Table I.

**Cell Culture and Transfection—**Human pancreatic Panc-1 and prostate PC-3 cancer cells (American Type Culture Collection, Manassas, VA) were maintained in Dulbecco’s modified minimal essential medium (DMEM) containing 10% fetal bovine serum (FBS). 1 day prior to transfection, 3 × 10<sup>5</sup> cells were seeded into 6-well plates. Cells were

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1 The abbreviations used are: DMEM, Dulbecco’s modified minimal essential medium; FBS, fetal bovine serum; HA, hemagglutinin; MTI, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline; TGFβ, transforming growth factor β; TGFβRI, TGFβ type II receptor; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end-labeling.
transfected with 2.5 μg each of expression construct using Cytofectene (Bio-Rad Laboratories) following the manufacturer’s recommendation. Metabolic Labeling—HEK293 cells (American Type Culture Collection) were transfected with 5 μg of plasmid DNA per 100-mm dish. After transfection, cells were grown in 10% PBS/DMEM, 80 μg/ml [14C]palmitic acid, and 5 mM sodium pyruvate for 48 h (19). Cells were washed twice with phosphate-buffered saline (PBS) and lysed with 1% Triton X-100, 50 mM Tris-Cl (pH 8.0), 150 mM NaCl, 0.02% sodium azide, 100 μg/ml phenylmethylsulfonylfluoride, and 1 μg/ml aprotinin. Lysates were collected and centrifuged for 10 min (12,000 × g) at 4 °C.

Colonies were visualized using a fluorescence microscope (Leica Microsystems, Bannockburn, IL), and pictures were taken with a digital camera (Diagnostic Instruments, Sterling Heights, MI).

Western Blot Analysis—Two days post-transfection, cells were washed once with cold PBS and lysed in 30 ml of reporter lysis buffer (Promega, Madison, WI). For AP-1 activity analysis, cells were harvested 48 h later and lysed in 200 μl of reporter lysis buffer (Promega, Madison, WI). For AP-1 activity analysis, 24 h after transfection cells were washed once with DMEM and incubated in DMEM supplemented with 0.5% PBS during the next 16 h before harvesting and lysis. Aliquots of cell extracts were assayed for β-galactosidase and luciferase activities.

Localization—Two 106 HEK293 cells were seeded onto coverslips in 6-well dishes. Two days post-transfection cells were fixed with 4% paraformaldehyde, permeabilized, and blocked with 0.2% Triton X-100 and 10% PBS in PBS. Transfected cells were stained with anti-HA antibody (Roche Diagnostics). Following three washes with PBS, the cells were incubated with a 1:250 dilution of fluorescein isothiocyanate-conjugated anti-mouse IgG secondary antibody (Sigma-Aldrich). Cells were visualized using a fluorescence microscope (Leica Microsystems, Bannockburn, IL), and pictures were taken with a digital camera (Diagnostic Instruments, Sterling Heights, MI).

In Vitro Translation/Prenylation—1 μg of each DNA construct was mixed with 25 μl of rabbit reticulocyte lysate (Promega) and 5 μCi of [3H]-labeled farnesyl diphosphate or [3H]-labeled geranylgeranyl diphosphate at 30 °C for 90 min. For determining the protein levels produced in the in vitro translation assay, 20 μCi of [35S]methionine were used instead of [3H]-labeled farnesyl diphosphate or [3H]-labeled geranylgeranyl diphosphate. The labeled proteins were then run on SDS-polyacrylamide gels and subjected to autoradiography. Gels were dried and subjected to autoradiography.

**FIG. 1. The ability of RhoB to suppress the promoter activity of p21waf, AP-1, and TGFβR requires cysteine 192.** A, sequence homology of RhoA and RhoB (asterisk designates residues that are different). B, expression levels of RhoB, RhoA, and mutagen constructs as measured by HA antibody Western immunoblotting and as described under “Experimental Procedures.” C–E. Panc-1 cells were cotransfected with luciferase under the control of TGFβIR, p21Waf and AP-1 promoters, respectively. In each experiment the luciferase activity was normalized for transfection efficiency against β-galactosidase activity and expressed as relative luciferase activity. Bars represent S.D. The data are representative of three independent experiments. *p values with respect to differences with RhoB (C), all p values < 0.005; D, p > 0.01; E, p < 0.01.

**Regulation of AP-1, p21waf, and TGFβR Receptor Promoter Activities—** Panc-1 cells were seeded at 3 × 105 cells per well in 6-well plates. 24 h later they were transfected with 0.5 μg of p21waf, TGFβ type II receptor (TGFβIR), or AP-1 promoter-luciferase constructs (21–23), 0.2 μg of pCMV-β-Gal, and 2 μg of each of RhoA, RhoB, mutants, or pcDNA3 vector using Cytofectene as indicated by the supplier. 15 h after transfection, the cells were replenished with fresh growth medium. For p21waf and TGFβ receptor promoter activity analysis, cells were harvested 48 h later and lysed in 200 μl of reporter lysis buffer (Promega, Madison, WI). For AP-1 activity analysis, 24 h after transfection cells were washed once with DMEM and incubated in DMEM supplemented with 0.5% PBS during the next 16 h before harvesting and lysis. Aliquots of cell extracts were assayed for β-galactosidase and luciferase activities.
Cysteine 192 Is Required for RhoB Tumor Suppression

Anchorage-independent Growth Assays—For soft agar growth assays, the transfected Panc-1 cells were seeded at 5000 cells per well in 6-well plates in 0.3% agar over a 0.6% agar layer as described previously (11). Cultures were fed once weekly until colonies grew to a suitable size (4 weeks) for observation. Colonies were photographed on a plate at 4 days of culture, the medium was replaced with 1 mg/ml MTT in the next day with 0.3% DMEM medium for 3 h. Me2SO was added to each well before absorbance at 540 nm was read on an automated microplate reader.

In Vitro Cellular Proliferation and TUNEL Assay—Two days post-transfection, cells were harvested by trypsinization and counted via cytrypan blue exclusion assay to determine cellular viability. Cells (10,000–25,000) were then spun onto glass slides using a Cytospin 3 centrifuge (Thermo Shandon, Pittsburgh, PA). After fixing the cells to the slides with 4% paraformaldehyde in PBS (pH 7.5) for 1 h at room temperature, cells were labeled for apoptotic DNA strand breaks by TUNEL reaction using an enzyme kit (Roche Diagnostics) according to the manufacturer’s instructions and then mounted in Vectashield mounting medium (Vector Laboratories, Burlingame, CA) containing 4,6-diamidino-2-phenylindole to counterstain DNA. Fluorescein-labeled DNA strand breaks (TUNEL-positive cells) were then visualized using a fluorescence microscope (Leica Microsystems), and pictures were taken with a digital camera (Diagnostic Instruments). TUNEL-positive nuclei were counted and compared with 4,6-diamidino-2-phenylindole-stained nuclei to determine the percentage induction of apoptosis.

RESULTS

RhoB/RhoA Site-directed Mutagenesis Screen of 27 Mutants Identifies Cysteine 192 as a Critical Amino Acid Required for RhoB Function—RhoA and RhoB share 86% amino acid sequence identity, yet RhoA promotes whereas RhoB suppresses tumorigenesis. To identify amino acids in RhoB that are critical to its tumor-suppressive activity, we first mutagenized amino acids 29, 100, 116, 123, 129, 140–143, 141, 146, 152, 154, 155, 173, 181, 183–187, 189, 190, 191, 192, and 193 in RhoB to the corresponding residues in RhoA (Table I). A RhoA–RhoB chimera was also made (Table I). The resulting 27 mutant proteins as well as wild type RhoB and RhoA were expressed in Panc-1 cells, and their effects on anchorage-independent growth as-measured by TGFβIIR promoter transcriptional activity (Fig. 1), shows that all HA-tagged RhoB mutants as well as wild type HA-RhoB and wild type HA-RhoA were expressed in Panc-1 cells, whereas pcDNA3 vector-transfected cells were devoid of HA-tagged proteins as expected. Fig. 1C shows that ectopic expression of wild type RhoB but not RhoA resulted in inhibition of TGFβIIR promoter transcriptional activity. Of the 27 mutant proteins generated, only those in which cysteine 192 or palmitoylation sites (189 and 192) in RhoB, has previously been shown to be important for RhoB function, as we had shown previously that RhoB promotes whereas RhoB suppresses tumorigenesis.

RhoB/RhoA Site-directed Mutagenesis Screen of 27 Mutants Identifies Cysteine 192 as a Critical Amino Acid Required for RhoB Function—RhoA and RhoB share 86% amino acid sequence identity, yet RhoA promotes whereas RhoB suppresses tumorigenesis. To identify amino acids in RhoB that are critical to its tumor-suppressive activity, we first mutagenized amino acids 29, 100, 116, 123, 129, 140–143, 141, 146, 152, 154, 155, 173, 181, 183–187, 189, 190, 191, 192, and 193 in RhoB to the corresponding residues in RhoA (Table I). A RhoA–RhoB chimera was also made (Table I). The resulting 27 mutant proteins as well as wild type RhoB and RhoA were expressed in Panc-1 cells, and their effects on TGFβIIR promoter transcriptional activity were determined. We used the TGFβIIR promoter/luciferase reporter as an initial screen for RhoB function, as we had shown previously that RhoB suppresses TGFβIIR promoter activity (Fig. 1B). Fig. 1B shows that all HA-tagged RhoB mutants as well as wild type HA-RhoB and wild type HA-RhoA were expressed in Panc-1 cells, whereas pcDNA3 vector-transfected cells were devoid of HA-tagged proteins as expected. Fig. 1C shows that ectopic expression of wild type RhoB but not RhoA resulted in inhibition of TGFβIIR promoter transcriptional activity. Of the 27 mutant proteins generated, only those in which cysteine 192 or cysteine 193 were mutated lost their ability to suppress TGFβIIR promoter transcriptional activity (Fig. 1C). Cysteine 193, the amino acid that is prenylated (both farnesylated and geranylgeranylated), has previously been shown to be important for RhoB function, whereas cysteine 192, one of the two palmitoylation sites (189 and 192) in RhoB, has not previously been implicated in RhoB function. Furthermore, whereas cysteine 192 was critical for inhibiting TGFβIIR transcriptional activity, cysteine 198, the other palmitoylation site, was not. mutating both cysteines 189 and 192 to serines also abolished the ability of RhoB to inhibit TGFβIIR promoter activity. Mutating cysteine 192 to either glycine (the corresponding residue...
Cysteine 192 Is Required for RhoB Tumor Suppression

in RhoA, alanine, or serine all resulted in loss of the ability of RhoB to inhibit TGFβIR promoter activity (Fig. 1C). In contrast, point mutations converting RhoB amino acids 29, 100, 116, 123, 129, 141, 146, 149, 152, 154, 155, 173, 181, and 191 to their corresponding amino acids in RhoA did not affect the ability of RhoB to inhibit TGFβIR promoter activity. The RhoA/RhoB chimera behaved like RhoB, not RhoA (Fig. 1C). Furthermore, replacing RTDD (amino acids 140–143) in RhoB by the corresponding KPEE residues in RhoA had no effect on RhoB activity (Fig. 1C). Similarly, replacing YGSQN (residues 183–187) in RhoB with the corresponding RGKKK residues in RhoA or with AGAAA did not affect RhoB activity. Moving palmitoylated cysteine 192 to position 190 also did not affect RhoB activity. Finally, deleting the tripeptide GCI, residues 188–190 in RhoB, which is absent in RhoA, did not affect the ability of RhoB to inhibit TGFβIR promoter activity (Fig. 1C).

Cysteine 192 Is Required for RhoB Inhibition of p21waf and AP-1 Promoter Transcriptional Activities—The results described above clearly demonstrate that the presence of cysteine 192 or cysteine 193 alone is insufficient and that they must both be present in RhoB in order for this protein to suppress TGFβIR promoter transcriptional activity. To further confirm the importance of these two amino acids in RhoB function, we evaluated the effects of the corresponding mutant proteins on p21waf and AP-1 promoter activities. Panels D and E in Fig. 1 show that ectopic expression of either wild type RhoB or the C189S/RhoB mutant resulted in inhibition of p21waf and AP-1 promoter activities. In contrast, the RhoA and RhoB point mutants C193S, C192S, C192A, C192G, and the double mutant C189S/C192S were unable to inhibit p21waf and AP-1 promoter activity. C192S and C193S Mutations Inhibit Palmitoylation and Mislocalize RhoB—We next determined the effect of cysteines 189, 192, and 193 on the palmitoylation and cellular localization of RhoB. As expected, mutating both cysteines 189 and 192 to serines resulted in a RhoB protein devoid of [14C]palmitic acid labeling (Fig. 2A). Furthermore, mutating one of these two cysteines at a time resulted in half of the [14C]palmitic acid labeling as being on wild type RhoB, which contains both cysteines. Mutating cysteine 193 to serine prevented palmitoylation, suggesting that prenylation of cysteine 193 is required for palmitoylation of cysteines 189 and 192 to occur. Finally, as expected, RhoA, which is known to lack palmitoylation sites, was not found to contain any [14C]palmitic acid label (Fig. 2A).

We next determined the effects of the mutations on the cellular localization of RhoB. Fig. 2B shows that wild type RhoB showed a punctate profile consistent with the previously reported endosome localization (19), whereas RhoA showed a diffused profile and was predominantly cytosolic. Furthermore, C192S and C193S RhoB mutants localized to the cytosol, whereas the C189S mutant showed a punctate profile similar to that of wild type RhoB (Fig. 2B).

C192S Mutation Does Not Affect Prenylation of RhoB—There are conflicting reports on the importance of cysteine 192 on the prenylation status of cysteine 193. Whereas Adamson et al. (19) reported that the cysteine 192 RhoB mutant becomes mainly farnesylated, Armstrong et al. (24) found that the cysteine 192 mutant was both farnesylated and geranylgeranylated. Using in vitro translation/prenylation assays, we demonstrated that C192S RhoB mutant was farnesylated and geranylgeranylated as efficiently as wild type RhoB (Fig. 2C).

Palmitoylated Cysteine 192 Is Required for RhoB to Inhibit Anchorage-dependent and Anchorage-independent Tumor Growth—Figs. 1 and 2 demonstrated that point mutations at one palmitoylation site (cysteine 192) but not the other (cysteine 189) resulted in a RhoB protein mutant that was mislocalized and unable to carry out its signaling function, thus leading to down-regulation of the transcriptional activities of TGFβIR, AP-1, and p21waf promoters. We next determined the effects of the point mutations on RhoB tumor-suppressive...
activity. Fig. 3A shows that ectopic expression of wild type RhoB as well as the RhoA/B chimera, but not RhoA, inhibited proliferation (anchorage-dependent tumor growth) of PC3 tumor cells by 40%. In contrast, ectopic expression of the point mutants C193S, C192S, C192A, and C192G, but not C189S, abolished the ability of RhoB to inhibit PC3 tumor cell proliferation. We next determined the effects of the mutations on the ability of RhoB to inhibit Panc-1 tumor cell colony formation on plastic. Fig. 3B shows that wild type RhoB (137 ± 32 colonies) and point mutant C189S (146 ± 27 colonies) inhibited Panc-1 colony formation as compared with vector control (426 ± 23). In contrast, RhoA (663 ± 84 colonies) as well as the RhoB point mutants C192S (538 ± 68 colonies) and C193S (647 ± 97 colonies) increased Panc-1 colony formation. Similarly, Fig. 3C shows that wild type RhoB (39 ± 17) and RhoB point mutant C189S (94 ± 36 colonies), but not RhoA (469 ± 62 colonies), RhoB point mutants C192S (562 ± 78 colonies), and C193S (543 ± 89 colonies), inhibited soft agar colony formation (anchorage-independent tumor growth) relative to vector control (684 ± 117 colonies).

Palmitoylated Cysteine 192, but Not Palmitoylated Cysteine 189, Is Required for RhoB to Induce Apoptosis—The Fig. 3 results discussed above show that the palmitoylated site of one cysteine (192) but not the other (189), along with the prenylation site cysteine 193, are critical for RhoB tumor cell growth. Because RhoB is also known to induce programmed cell death (apoptosis), we next determined the importance of cysteines 192 and 193 on the ability of RhoB to induce apoptosis by TUNEL staining. Panels A and B of Fig. 4 show that vector-transfected cells contained 10 ± 2% apoptotic cells, whereas cells transfected with RhoB, C189S RhoB, or the RhoA/B chimera mutant contained 19 ± 2%, 14 ± 2%, and 17 ± 1% apoptotic cells, respectively. In contrast, cells transfected with RhoA and the point mutants C193S or C192S contained only 4 ± 1%, 2 ± 0.4%, or 4 ± 0.5% apoptotic cells, respectively. Therefore, C192S and C193S mutations blocked the ability of RhoB to induce apoptosis.

DISCUSSION

In this work we mutated 29 amino acids in RhoB to the corresponding residues in RhoA to identify those residues critical to the ability of RhoB to inhibit malignant transformation. We found that changing cysteine 192 in RhoB to the corresponding glycine in RhoA abolished the ability of RhoB to regulate transcription and to inhibit tumor proliferation, colony formation, and soft agar growth, as well as to induce apoptosis. In contrast, point mutations converting RhoB amino acids 29, 100, 116, 129, 141, 146, 149, 152, 154, 155, 173, 181, and 191 to their corresponding amino acids in RhoA did not affect RhoB function. Furthermore, replacing RTDD (amino acids 140 to 143) in RhoB with the corresponding KPEE of RhoA had no effect on RhoB activity. Similarly, replacing YGSQN (residues 183–187) in RhoB with the corresponding RGKKK of RhoA or AGAAA did not affect RhoB activity. A RhoA-(1–180)/RhoB-(181–196) chimera was as efficient as RhoB in repressing TGFβ type II receptor promoter activity.
and inhibiting cell proliferation as well as in inducing apoptosis. Cysteine 192 is one of the two amino acids in RhoB that are posttranslationally modified with palmitic acid, and this palmitoylation has been suggested previously to be critical for the localization of RhoB to the endosomes (19). Consistent with these findings are our results showing that the C192S mutant inhibited palmitoylation and was mislocalized. In contrast to cysteine 192, we have shown that cysteine 189, the other palmitoylation site, is not critical for the localization or function of RhoB. Furthermore, the deletion in RhoB of the tripeptide GCI (188–190), a peptide sequence that is absent in RhoA, did not affect RhoB function. Finally, moving cysteine 192 to position 190 did not alter RhoB function either. These results indicate that palmitoylated cysteine 192 but not cysteine 189 is required for RhoB function. We also found that cysteine 193, the site of RhoB that is prenylated by either a farnesyl or a geranylgeranyl group, is also required for the ability of RhoB to suppress transcription, inhibit proliferation and transformation, and induce apoptosis. This is consistent with previous reports that showed that mutating cysteine 193 to serine 193 or deleting the last four amino acids of RhoB, CKVL (193–196), results in loss of RhoB function (8, 25). These results demonstrate that both (but not each alone) palmitoylated cysteine 192 and prenylated cysteine 193 must be present in RhoB for its tumor-suppressive and apoptotic activities.

Previous work has demonstrated that cysteine 192 is critical for the proper cellular localization of RhoB (19). However, there are no reports on the importance of this amino acid for the ability of RhoB to regulate transcription, malignant transformation, and apoptosis. Furthermore, there are conflicting reports on the importance of cysteine 192 on the prenylation of cysteine 193. Adamson et al. (19) showed that cysteine 193, which can be either farnesylated or geranylgeranylated in wild type RhoB, becomes mainly farnesylated in a mutant RhoB when the C-terminal five amino acids CCKVL (192–196) were changed to SCKVL. One interpretation given by the authors of this study was that cysteine 192 is critical to prenylation and that the double cysteine CC (192 and 193) is an important recognition site for geranylgeranyltransferase type I to transfer the geranylgeranyl group to cysteine 193. In contrast to this study, Armstrong et al. (24) found that cysteine 192 was not important to the prenylation of cysteine 193 and that the C192S RhoB mutant was as efficiently farnesylated or geranylgeranylated as wild type RhoB. Our studies confirm those of Armstrong et al. (24). Also, consistent with this was a report from Baron et al. (26) demonstrating that RhoB prenylation on cysteine 193 is only driven by the nature of the three carboxyl-terminal KVL (194–196) residues. It is clear from our present study as well as from previous studies that a RhoB mutant lacking palmitoylated cysteine 192, but not 189, is unable to localize properly. What is even more important is that this mutant is inactive in its ability to suppress malignant transformation and induce apoptosis even though it is still prenyl-
ated. This finding, coupled with the fact that the RhoA/RhoB chimera behaves like RhoB not like RhoA, leads us to conclude that one of the major reasons for the opposing effects of RhoA and RhoB on malignant transformation may reside, at least in part, in their different cellular localizations, from which they affect different effectors.

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