It has recently been reported that Ki-Ras protein can be modified in vitro by farnesylation or geranylgeranylation. However, a previous analysis of Ki-Ras prenylation in vivo found only farnesylated Ki-Ras. In this report it is shown that under normal conditions, Ki-Ras is farnesylated in vivo and that its prenylation in cells is inhibited by a GGTase I inhibitor B956 or B957, farnesylation is inhibited and Ki-Ras becomes geranylgeranylated in a dose dependent manner. These results have strong implications in the design of anticancer drugs based on inhibition of prenylation.

Post-translational modifications of small molecular weight GTP-binding proteins have been the subject of intense studies since the first reports of prenylation appeared (1–3). Many of these proteins, and specifically the Ras proteins, are involved in tumorigenesis. Since prenylation is necessary for the transformation potential of Ras proteins, a number of groups have been dedicated to the design of inhibitors of prenylation as a strategy to inhibit tumor formation (4).

Two types of prenyl modifications have been described: farnesylation and geranylgeranylation. The enzymes FTase and GGTase I recognize a tetra peptide sequence at the C terminus of a protein (5, 6). This sequence has been designated the CAAX box, and it determines whether a protein will be prenylated (7). In this sequence, C stands for cysteine and A for any amino acid. The amino acid X specifies whether the protein will be farnesylated (methionine or serine) or geranylgeranylated (leucine or phenylalanine) by the appropriate enzyme. This rule is followed in the majority of cases. However, recent reports have shown that Ki-Ras, whose C terminus CVIM predicts farnesylation, can also be geranylgeranylated in vitro (8), and that its prenylation in cells is inhibited by a GGTase I inhibitor (9). Since activated Ki-Ras is the type of Ras most frequently found in human cancers, it is very important to determine how it is modified in vivo and whether this modification can be inhibited.

Cell labeling with [3H]mevalonate in the presence of inhibitors of hydroxymethylglutaryl-CoA reductase such as lovastatin allows the radioactive labeling of cholesterol and its intermediate isoprenoids. However, a major drawback of this approach is the low level of [3H]mevalonate incorporation into cells. The fortuitous identification of the gene for a mevalonate transport membrane protein (pMev) has provided a tool that makes the identification of isoprenoid and non-steroidal isoprenoid intermediates easier (10, 11). Transfection of cells expressing either Ha-Ras or Ki-Ras oncogenes with the mevalonate transporter and growth of the cells under conditions in which the endogenous pools of farnesyl diphosphate and geranylgeranyl diphosphate are radioactively labeled enabled us to demonstrate that both Ha-Ras and Ki-Ras are farnesylated under normal conditions. In the presence of the farnesyl transferase inhibitor B956, farnesylation of both Ha-Ras and Ki-Ras was inhibited in a dose-dependent manner. However, as farnesylation was inhibited, Ki-Ras became geranylgeranylated. These results have strong implications for the design of prenylation inhibitors as potential anticancer agents.

**EXPERIMENTAL PROCEDURES**

**Materials**—The inhibitors B956 and B957 were synthesized at Eisai Research Institute. Lovastatin was kindly provided by Dr. A. Alberts, Merck Research Laboratories. pMev cDNA was obtained from ATCC. [3H]Mevalonolactone (60 Ci/ mmol, ABC) was routinely dried and resuspended in sterile water at 10 mM. When specified, the lactone was converted to the acid by heating at 37 °C with 1 eq of NaOH. After 30 min, the sample was neutralized with HCl, sterile-filtered, and stored at 4 °C.

**Purification of Recombinant Proteins**—Ha-Ras and Ha-Ras(CAIL) were purified as described previously (13). Ki-Ras(12V) and GST-Rho A(V14) were purified from BL21(DE3)pLysS cells. Briefly, after transfection, competent BL21(DE3)pLysS cells were plated and grown overnight at 30 °C. Single colonies were picked and grown in LB plus antibiotics at 30 °C until absorbance at 600 nm was 0.5, at which time the cultures were kept at 4 °C overnight. Next day, the bacterial cultures were centrifuged and the clear supernatant was applied to a Resource-S column (Pharmacia) equilibrated in Buffer A and eluted with a 0–50% linear gradient of Buffer B (Buffer A plus 1M NaCl). The dialyzed material containing Ki-Ras was dialyzed against Buffer B and purified with GGTase I and GGTase II.

**In Vitro Prenylation**—Ki-Ras (0.06 mg/ml) was flash-frozen into lysis buffer, pH 8.0, 10 mM MgCl2, 1 mM EDTA, and 1 mM dithiothreitol. The purified material containing Ki-Ras was dialyzed against Buffer B and purified with GGTase I and GGTase II.

**Rho A-glutathione S-transferase fusion protein** was purified by binding to glutathione-Sepharose 4B (Pharmacia Biotech Inc.) according to supplier’s instructions. After washing beads to remove unbound material, Rho A was eluted with thrombin (Pharmacia). The fractions containing Rho A were co-purified with GST-Rho A(V14) on a Resource-S column (Pharmacia) equilibrated in Buffer B and eluted with a 0–50% linear gradient of Buffer B (Buffer B plus 1 M NaCl). The fractions containing Ki-Ras were pooled, and octyl glucoside was added to 0.2% and Ki-Ras (0.06 mg/ml) was flash-frozen and stored at −80 °C.

To purify soluble Ki-Ras, 1 mM phenylmethylsulfonaryl fluoride was added to the defrosted lysate; this was clarified by sonication, followed by centrifugation to remove debris. The clear supernatant was applied to a Resource-S column (Pharmacia) equilibrated in Buffer B and eluted with a 0–50% linear gradient of Buffer B (Buffer B plus 1 M NaCl). The fractions containing Ki-Ras were pooled, and octyl glucoside was added to 0.2%. Ki-Ras (0.06 mg/ml) was flash-frozen and stored at −80 °C.

Rho A-glutathione S-transferase fusion protein was purified by binding to glutathione-Sepharose 4B (Pharmacia Biotech Inc.) according to supplier’s instructions. After washing beads to remove unbound material, Rho A was eluted with thrombin (Pharmacia) and thrombin was removed with p-amino-phenylbenzamidine beads. Beads were sedimented by centrifugation, and cleaved, purified, soluble Rho A was recovered from the supernatant.

In **Vivo Prenylation**—To standardize prenyl cysteine identification, Ki-Ras (29 pmol), Rho A (78 pmol), or Ha-Ras(CAIL) (26 pmol) was...
prenylated in medium containing [3H]FPP or [3H]GGPP as described previously (13), except that the incubation continued for 2 h. The labeled proteins were trichloroacetic acid-precipitated, washed with 90% acetone, and digested with Pronase, followed by prenyl group extraction as described below. In vitro inhibition of prenylation by B957 was determined using recombinant Ha-Ras or Ha-Ras(CAL) substrates (13).

Transfection of pMev into Ras-expressing Cells—NIH-3T3 cells overexpressing either Ha-Ras (zH1 cells obtained from C. Der, University of North Carolina) or Ki-Ras (DK1 cells, Ref. 12) were transfected with 2 μg of pMev DNA using LipofectAMINE according to the manufacturer’s protocol (Life Technologies, Inc.). Cells were selected in DMEM, 10% calf serum, 20 mM mevalonate for approximately 6 h. DMEM containing 10% calf serum, 50 μM mevalonate for approximately 6 h. DMEM containing 10% calf serum, 50 μM lovastatin (selection/maintenance medium). After 15 days under selection, clones were isolated and maintained in selection medium. To assess the functional expression of pMev, the uptake of [3H]mevalonate was determined by hexane:propanol extraction of cell lysates (11). Alternatively, [3H]mevalonate incorporation into protein was measured after trichloroacetic acid precipitation from cell lysates. Mevalonate Labeling—For isotope labeling, cells were plated at a density of 2 × 10^4 cells/10-cm plate and grown overnight in maintenance medium (see above). Prior to adding label, cells were starved of mevalonate for approximately 6 h in 4.5 ml of DMEM containing 10% calf serum, 50 μM lovastatin, with or without FTase inhibitor. After starvation, 0.5 ml of medium containing at least 750 μCi of [3H]mevalonate and 250 μCi of [3H]mevalonolactone to the concentrations indicated in figure legends was added. Cells were grown for 16 h and lysed in 0.5 ml of 1% Nonidet P-40, 0.5% sodium deoxycholate, 50 mM Tris-Cl, pH 8.0, 100 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 0.01 mM leupeptin and 0.01 mM pepstatin.

Prenyl Group Isolation and Characterization—Ras from [3H]mevalonate-labeled lysates was immunoprecipitated from comparable amounts of proteins with Y13–259 antibody. After washing, the protein A-agarose-Ras complex was resuspended in 250 μl of 100 mM Tris-Cl, pH 7.5, and 500 μl of Pronase (10 mg/ml in 100 mM Tris-Cl, pH 7.5, 100 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 0.01 mM leupeptin, and 0.01 mM pepstatin). Prenyl cysteine groups were extracted four times from the aqueous Pronase solution with 500 μl of chloroform:methanol (9:1). All four extractions were pooled into a siliconized glass tube, and the organic solvent was evaporated under a stream of nitrogen. Prenyl cysteines were resuspended in 150 μl of 20% acetonitrile:methanol containing 0.5% each F-Cys and GG-Cys standards (prepared by S-alkylation of cysteine with the corresponding isoprenyl halides).

F-Cys and GG-Cys were separated by reversed phase HPLC using a TosoHaa TSK-gel, ODS-120T (4.6 mm × 25 mm) column. Elution was performed with a 10-min linear gradient of 100% buffer A (10% acetonitrile, 0.15% trifluoroacetic acid) to 60% buffer B (100% acetonitrile, 0.15% trifluoroacetic acid), 15 min at 60% B, followed by a 15-min linear gradient to 100% B. The flow rate was constant at 1 ml/min. This protocol allowed for optimal separation of F-Cys and GG-Cys. One-ml fractions were collected, 10 ml of Liquisec (National Diagnostics) was added, and the samples were counted in a scintillation counter.

RESULTS

The pMev clone is available from ATCC in a mammalian expression vector with the neo<sup>B</sup> gene for selection. However, the cells of interest already express the neo<sup>R</sup> gene. A novel selection protocol was developed based partially on conditions already described to isolate the transporter (10). After transfection with pMev, the cells were grown in limiting concentrations of mevalonate: 50 μM lovastatin, 20 μM mevalonate. Under these conditions most of the control, untransfected cells died after a week, but many of the pMev-transfected cells survived and formed numerous colonies. A few of these clones were isolated and expanded. When examined for [3H]mevalonate uptake, up to 10-fold more 3H products were extracted from DKMev clones than from untransfected cells (Table I, DKMev clones). Alternatively, incorporation of [3H]mevalonate into trichloroacetic acid-precipitable protein was determined, in which case up to 60-fold more 3H was incorporated into zHMev clones than in controls (Table I, zHMev). In contrast, control cells that adapted to grow under limiting concentrations of mevalonate, accumulated as little mevalonate as untransfected cells (Table I, zH1 selected). Clones DKMev2 and zHMev6 were kept under limiting mevalonate conditions to maintain the expression of the transporter and used for further studies.

Since GGPP is not a major intermediate in the synthesis of cholesterol, it was important to determine whether the internal pool of GGPP could be efficiently labeled by radioactive mevalonate. Analysis of post-translational processing under maintenance conditions indicated that more than 90% of Ha-Ras was processed (data not shown), but only 36% of Rap 1A proteins were processed (Fig. 1, lanes 2–6). When cells were grown with increasing concentrations of mevalonolactone in the medium, the amount of processed Rap 1A protein increased (Fig. 1, lanes 2–6). At concentrations above 150 μM mevalonolactone, all the Ha-Ras (not shown) and Rap 1A were post-translationally processed, as indicated by the disappearance of the slower migrating band (Fig. 1, lanes 6). Analysis of prenyl groups from total protein extracted from cells incubated at increasing concentrations of [3H]mevalonolactone (Fig. 2) showed an increase in the total amount of prenylated cysteines, as well as a higher increase in GG-Cys in comparison to F-Cys. Thus, when cells were labeled at low mevalonolactone concentration (33 μM, Fig. 2) the amount of F-Cys was higher than GG-Cys (GG-Cys/F-Cys = 0.8). As the mevalonolactone concentration was increased, the amount of GG proteins also increased and the ratio GG-Cys/F-Cys became 1.8 at 250 μM (Fig. 2). These results are in agreement with previously published information, indicating that in the presence of hydroxymethylglutaryl-CoA reductase inhibitors like lovastatin, 20 μM mevalonate is enough for minimal synthesis of FPP and GGPP (14), while 200 μM mevalonate will maintain non-sterol isoprenoid synthesis as well (15). Since the cell culture medium contains serum, it is likely that no cholesterol synthesis is needed, and therefore all the FPP becomes available for prenylation of proteins as well as dolichol and ubiquinone synthesis. However, since GGPP is not a major precursor in the synthesis of cholesterol, it appears that not enough of it is made to prenylate all proteins. In other words, under the maintenance conditions described here, the endogenous pools of FPP and GGPP are practically depleted. Upon incubation with 250 μM [3H]mevalonolactone, the pools are reloaded but now with [3H]FPP and [3H]GGPP, which are then used for protein prenylation. Curiously, despite published data indicating that pMev does not transport mevalonolactone efficiently (10), no difference was found between the accumulation of radioactive lactone or acid after overnight incubations (data not shown).

Identification of the prenyl groups was simplified by the elimination of any alkylating or oxidative cleavage step. The protocol was optimized using recombinant, in vitro prenylated Ki-Ras, Rho A, or Ha-Ras(CAL). After in vitro prenylation, the proteins were digested with Pronase, the prenylated cysteines

### Table I

| Cell type           | Uptake | Incorporation |
|---------------------|--------|--------------|
| DKMev1              | 12,396 | 1602         |
| DKMev2              | 16,961 | 1658         |
| DKMev3              | 7797   | 1815         |
| DKMev4              | 8761   | 1965         |
| DKMev5              | 3854   | 4290         |
| DKMev6              | 9851   | 72           |
| zH1                 | 1595   | 87           |

<sup>a</sup> Uptake of [3H]mevalonate determined by hexane:propanol extraction of whole cell lysates.

<sup>b</sup> Incorporation of [3H]mevalonate into trichloroacetic acid-precipitable protein from whole cell lysates.

DK1 and zH1 are the parental cell lines. zH1 (selected) correspond to zH1 cells grown under selection conditions.
extracted into an organic phase, and then separated by HPLC. In the case of farnesylated Ki-Ras, a single peak of radioactivity at 23 min, coeluting with the F-Cys standard, was obtained (Fig. 3A, upper trace). For geranylgeranylated Ki-Ras, two peaks of radioactivity were observed, a major one at 36 min coeluting with GG-Cys standard and a minor peak eluting earlier, at 28 min (Fig. 3A, lower trace). To confirm this pattern, two normally geranylgeranylated proteins, Rho A and Ha-Ras (CAIL) were labeled in vitro with [3H]GGPP and their prenylated peptides analyzed by HPLC. As seen in Fig. 3B, these two proteins present the same profile, namely a major peak at 36 min coeluting with GG-Cys standard and a smaller peak at 28 min. We have observed that the magnitude of the 28-min peak increases and the 36-min peak decreases with longer incubations in Pronase (data not shown). This suggests that the 28-min peak is a degradation product of GG-Cys, perhaps an oxide. To test this idea, the sulfoxide of GG-Cys was chemically synthesized, and it was found that, in fact, it elutes from HPLC at 28 min (data not shown). Nevertheless, the elution pattern of GG-Cys is very diagnostic regardless of whether the sample is derived from recombinant proteins prenylated in vitro or from whole cell lysate immunoprecipitation (see below).

To identify Ki-Ras prenyl modification in vivo, DKMeV cells were grown in [3H]mevalonolactone in the presence or absence of the FTase inhibitor B956 as described under “Experimental Procedures.” In the absence of inhibitor, there is a single peak of radioactivity at 23 min, coeluting with F-Cys standard (Fig. A). In the presence of B956 from 0.2 to 100 μM, peaks corresponding to GG-Cys appear (Fig. 4A). The radioactivity associated with F-Cys decreases significantly, in a dose-dependent manner, from 2 μM to 100 μM B956 treatment (Fig. 4A). In contrast, the radioactivity associated with the two peaks of GG-Cys at 28 and 36 min increase at the same concentrations of B956 (Fig. 4A). Since the specific activity of the F and GG moieties is different, and the pools of FPP and GGPP are not known, it is not possible to obtain quantitative data from this experiment. Nevertheless, the appearance of a significant amount of GG-Cys in the presence of the inhibitor is indisputable.

In contrast to the above data, when the prenylation of Ha-Ras was analyzed, only a peak corresponding to F-Cys was observed (Fig. 4B). The amount of farnesylated Ha-Ras decreased with increasing concentrations of B956 (Fig. 4B). In agreement with previous data (12), the farnesylation of Ha-Ras was more sensitive than Ki-Ras to inhibition by B956. In fact, at 0.2 μM B956 about half as much radioactivity as in the control eluted as F-Cys. Almost no Ha-Ras was farnesylated at 2 μM B956 (Fig. 4B). In contrast to Ki-Ras, no evidence that Ha-Ras can be geranylgeranylated was observed even at 100 μM B956. We previously reported that 0.5 μM B956 inhibits post-translational modification of Ha-Ras by 50% (12). Thus, both Western blot (12) and HPLC (this work) identification methods give comparable results for inhibition of Ha-Ras prenylation. We also reported that Ki-Ras prenylation was inhibited by 50% at 25 μM B956 (12). However, this value was obtained by inhibition of [3H]mevalonate incorporation into Ki-Ras at low mevalonate concentration, where not enough GGPP was being synthesized. In view of the data presented here, that result is not valid. Unfortunately the correct concentration of B956 that inhibits Ki-Ras prenylation by 50% can not
be determined here.

B956 is not a very specific FTase inhibitor in vitro. It inhibits H-CAIL geranylgeranylation and Ha-Ras farnesylation equally with an IC50 of 20 nM. In contrast, B957, the (trans, trans) isomer of B956, has similar potency against FTase, but it is more specific for FTase than GGTase in vitro with IC50 values of 40 nM (FTase) and 460 nM (GGTase I). The effect of B957 on Ki-Ras prenylation was also tested (Fig. 5). In this case, a similar inhibition of farnesylation was observed when compared with the effect of B956. Peaks corresponding to GG-Cys appeared after treatment with 2 μM or more B957, concurrent with a decrease in the F-Cys peak (Fig. 5). However, there seems to be more GG-Cys formation than when the cells were treated with B956. This is more evident if the ratios of radioactivity associated with the F-Cys and GG-Cys peaks are compared. Thus, at 2 μM either B956 or B957, the ratio F-Cys/GG-Cys is 0.9. When the concentration of inhibitor is increased to 20 μM, the F-Cys/GG-Cys ratio becomes 0.5 for B956 treatment and 0.26 for B957. At the highest concentration tested, 100 μM, the ratio is 0.3 for B956 and 0.1 for B957. These results indicate that B957 inhibits Ki-Ras farnesylation at least as efficiently as B956. However, B956 seems to inhibit both farnesylation and geranylgeranylation of Ki-Ras, while B957 inhibits only farnesylation.

**DISCUSSION**

Conventional techniques were used to determine the identity of the prenyl group attached to Ras proteins, but they were modified with simpler protocols. Important modifications were made to the protocols to simplify the analysis of prenyl group identity.

**FIG. 4.** Identification of the prenyl groups present in Ki-Ras or Ha-Ras in the presence or absence of B956. DKMev 2 (A) or zHMev 6 (B) cells were grown in media containing 225 μM [3H]mevalonolactone and 0, 0.2, 2, 20, or 100 μM B956. After overnight incubation, the proteins were immunoprecipitated, and the prenyl groups were extracted and separated by HPLC as indicated under “Experimental Procedures.” The position of authentic farnesyl-cysteine and geranylgeranyl-cysteine standards is indicated. The presence of B956 in DKMev cells resulted in a GG-Cys peak and the extra peak associated with GG-Cys at 28 min. No GG-Cys peaks are observed from Ha-Ras samples at any concentration of B956. However, sometimes extraneous radioactivity eluting later than 36 min is observed (2B, 0.2 μM). These experiments have been repeated at least twice.

**FIG. 5.** Effect of B957 on Ki-Ras prenylation. DKMev 2 cells were treated overnight with 0, 2, 20, or 100 μM B957 in the presence of 275 μM [3H]mevalonolactone. The extracted prenyl groups were analyzed by HPLC as described. The elution position of F-Cys and GG-Cys standards is indicated.
the selection and maintenance of cells at very low mevalonate concentration and the identification of prenyl cysteines without cleavage of the prenyl group (1, 16). This latter modification eliminated technical difficulties and considerably shortened the time required for prenyl identification. It did introduce an artifact, the oxidation of GG-Cys, but since its presence depends on the length of incubation in Pronase, carefully controlling the conditions gives consistently reproducible results. On the other hand, selection of cells transfected with the pmEv transporter in lovastatin and low mevalonate concentration allows the introduction of the transporter into any cell, regardless of whether they have been previously transfected. However, when cells were kept in 20 μM mevalonate, a large fraction of prenylated proteins were not post-translationally processed (Figs. 1 and 2). Despite the presence of non-prenylated proteins, the appearance and growth properties of the cells were apparently normal. This finding is surprising and indicates that either very little of these proteins is required for survival or that the prenylated proteins analyzed here are not necessary for cell growth.

It has been previously reported in the literature that Ki-Ras is farnesylated in vivo (1). The work presented here confirms this finding, but it shows for the first time that Ki-Ras can also be geranylgeranylated in vivo. Under normal conditions, Ki-Ras is exclusively farnesylated. However, upon treatment of cells with FTase inhibitors like B956 and B957, in vivo farnesylation is inhibited and Ki-Ras is alternatively modified to a significant extent by geranylgeranylation. This effect is probably true for many FTase inhibitors, as indicated by their reduced effect on Ki-Ras transformed cells (12, 17). In vitro data have indicated that Ki-Ras is a substrate for both FTase and GTase I (8). However, the affinity of Ki-Ras for FTase is much higher than that for GTase, suggesting that Ki-Ras will be preferentially farnesylated in vivo (8). We demonstrated here that this prediction is valid and that only when the conditions are altered, as in the presence of FTase inhibitors, does Ki-Ras become geranylgeranylated. Perhaps Ki-Ras plays a crucial role in maintaining cell growth and its alternative prenylation is a protective mechanism developed by the cells.

Previous to this study, a major question has been whether it is possible to detect Ki-Ras-GG in cells. Reports by others have suggested that this might not be possible because labeling must be done under limiting concentrations of mevalonate (17). Under these conditions, geranylgeranylation of proteins is reduced compared with farnesylation (Ref. 14 and Figs. 1 and 2). However, when the cells were transferred to higher mevalonolate concentration, the synthesis of FPP and GGPP was stimulated and normal protein prenylation resumed. Even though the specific activities of [3H]FPP and [3H]GGPP were lower than under limiting conditions, enough [3H]mevalonate was incorporated to adequately label FPP and GGPP. It should be mentioned that the reported Km for mevalonate uptake by pmEv is 300 μM (10). Therefore, at the concentrations used here for cell labeling (250 μM), significantly more mevalonate is taken up than under limiting conditions (20 μM), counterbalancing the lower specific activity.

The data presented here are in disagreement with a recently published article suggesting that Ki-Ras is normally geranylgeranylated (9). This suggestion was based on data showing that Ki-Ras post-translational modification and signaling were inhibited by a specific GTase I inhibitor, GTGI-286. We cannot explain this discrepancy. It is possible that the inhibitor used, although not very effective against Ha-Ras farnesylation, inhibits Ki-Ras farnesylation as efficiently as geranylgeranylation. Data on inhibition of Ki-Ras prenylation by GTGI-286 in vitro were not presented (9). It will be important to identify the type of prenyl group modifying Ki-Ras in the presence of GTGI-286 to clarify this issue.

Inhibition of post-translational processing of members of the Ras family has become very popular in recent years, because of the possibility of using the inhibitors as anticancer drugs (4, 18). Up to this point, the emphasis has been on designing very specific inhibitors of FTase to avoid possible toxic effects originating from inhibition of the related GTase I enzyme (19–21). Thus, most compounds synthesized so far have been good inhibitors of Ha-Ras-transformed cells and not very potent against Ki-Ras-transformed cells. The lack of potency of FTase inhibitors against Ki-Ras-transformed cells could be explained by a failure to inhibit Ki-Ras post-translational processing by GTase I. However, this explanation implies that Ki-Ras is the only factor controlling cell growth, and present evidence suggests that this might not be true. Although it has been shown that Ki-Ras-GG binds to SOS, a guanine nucleotide exchange factor (22), it remains to be studied whether Ki-Ras-GG is functional in downstream events and whether it has the same role in cell growth as farnesylated Ki-Ras. The data presented here suggest that it is very important to further characterize the properties and functions of Ki-Ras. Nevertheless, the information gathered so far imply that it would be better to design prenylation inhibitors that can inhibit both farnesylation and geranylgeranylation of Ki-Ras for development as potential cancer drugs.

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