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# Combination of the novel farnesyltransferase inhibitor RPR130401 and the geranylgeranyltransferase-1 inhibitor GGTI-298 disrupts MAP kinase activation and G<sub>1</sub>-S transition in Ki-Ras-overexpressing transformed adrenocortical cells

Jean-Luc Mazet<sup>a,1</sup>, Martine Padieu<sup>a,1</sup>, Hanan Osman<sup>a</sup>, Gabrielle Maume<sup>a</sup>, Patrick Mailliet<sup>b</sup>, Norbert Dereu<sup>b</sup>, Andrew D. Hamilton<sup>d</sup>, François Lavelle<sup>b</sup>, Saïd M. Sebtic<sup>c</sup>, Bernard F. Maume<sup>a,\*</sup>

<sup>a</sup> *Unité Mixte de Recherche INRA-Université de Bourgogne, Phytopharmacie et Biochimie des Interactions Cellulaires, INRA, BV 1540, 21034 Dijon, Cedex, France*

<sup>b</sup> *Centre de Recherche Rhône-Poulenc Rorer, B.P. 14, 94403 Vitry-sur-Seine, Cedex, France*

<sup>c</sup> *Drug Discovery Program, H. Lee Moffitt Cancer Center, Department of Biochemistry and Molecular Biology at the University of South Florida, Tampa, FL 33612, USA*

<sup>d</sup> *Department of Chemistry, Yale University, New Haven, CT, USA*

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**Abstract** To test the Kirsten-Ras (Ki-Ras) alternative prenylation hypothesis in malignant transformation, we used a novel farnesyltransferase inhibitor competitive to farnesyl-pyrophosphate, RPR130401, and a CaaX peptidomimetic geranylgeranyltransferase-1 inhibitor GGTI-298. In Ki-Ras-overexpressing transformed adrenocortical cells, RPR130401 at 1–10  $\mu$ M inhibited very efficiently the [<sup>3</sup>H]farnesyl but not [<sup>3</sup>H]geranylgeranyl transfer to Ras. However, proliferation of these cells was only slightly sensitive to RPR130401 (IC<sub>50</sub> = 30  $\mu$ M). GGTI-298 inhibited the growth of these cells with an IC<sub>50</sub> of 11  $\mu$ M but cell lysis was observed at 15  $\mu$ M. The combination of 10  $\mu$ M RPR130401 and 10  $\mu$ M GGTI-298 inhibited efficiently (80%) cell proliferation. These combined inhibitors but not each inhibitor alone blocked the cell cycle in G<sub>0</sub>/G<sub>1</sub> and disrupted MAP kinase activation. Thus, combination of two inhibitors, at non-cytotoxic concentrations, acting on the farnesyl-pyrophosphate binding site of the farnesyltransferase and the CaaX binding site of the geranylgeranyltransferase-1 respectively is an efficient strategy for disrupting Ki-Ras tumorigenic cell proliferation.

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**Key words:** Kirsten-Ras; Geranylgeranyltransferase; Farnesyltransferase; Prenylation; Alternative pathway; Anti-proliferative effect

## 1. Introduction

Over the last decade, protein prenylation has been a focus

of many scientific investigations because several proteins involved in cell cycle control and differentiation, including Ras proteins, are post-translationally modified by the isoprenoids farnesyl and geranylgeranyl [1]. Furthermore, prenylation of oncogenic Ras proteins is required for their tumorigenesis [2]. Mammalian cells express three types of *ras* genes (Ha-, N- and Ki-*ras*) [3]. About 30% of all human cancers express mutated Ras proteins, the most prevalent form being of the Ki-type [4]. Translocation of Ras p21 to the plasma membrane due to post-translational modifications was shown to be essential for its function [1]. Inhibition of prenylation of mutated Ras results in an accumulation of a cytosolic protein which does not have a tumorigenic function. Two different prenyl groups, farnesyl (C<sub>15</sub>) and geranylgeranyl (C<sub>20</sub>), were found to be attached covalently to the cysteinyl residue at the carboxyl-terminus of proteins that end in a CaaX motif (C = Cys, a = aliphatic residue and X = an aminoacyl residue such as Ser, Met or Leu). Prenyl transfers are catalyzed by protein farnesyltransferase (FTase), preferring Ser or Met, and protein geranylgeranyltransferase-1 (GGTase-1), preferring Leu at the X position [1]. Farnesylation is the main prenylation of Ras proteins and several FTase inhibitors were shown to block selectively processing and signaling of oncogenic Ha-Ras [5]. Nevertheless, oncogenic Kirsten-Ras (Ki-Ras) B prenylation is less sensitive to FTase inhibitors [6]. It was reported that the Ki-RasB protein can be geranylgeranylated by GGTase-1 in vitro [6] and that its prenylation was disrupted in whole cells by a potent inhibitor of GGTase-1 [7]. In a previous study, we showed that overexpressed Ki-Ras protein was geranylgeranylated as well as farnesylated in *ras*-transformed adrenocortical (RTAC) cells [8]. Other studies have shown that Ki-RasB was geranylgeranylated only when cells were treated with FTase inhibitors [9,10]. Our and other findings suggest that the Ki-Ras protein is prenylated by an alternative pathway using GGTase-1. Geranylgeranylation of Ras occurs when either FTase is blocked by FTase inhibitors, deprived from the farnesyl-pyrophosphate (FPP) substrate or saturated by an excess of Ras proteins. Whether geranylgeranylated Ras is as efficient as farnesylated Ras at inhibiting cell proliferation is not yet known. The discovery of the novel potent FTase inhibitor benzoperhydroi-

\*Corresponding author. Fax: (33) (380) 693265.  
E-mail: maume@chassagne.epoisses.inra.fr

<sup>1</sup> Both authors contributed equally to this work and should be considered first authors.

**Abbreviations:** BPHI, benzoperhydroisindole; FPP, farnesyl-pyrophosphate; FTase, farnesyltransferase; GGPP, geranylgeranyl-pyrophosphate; GGTase, geranylgeranyltransferase; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; MAP, mitogen-activated protein; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RTAC, *ras*-transformed adrenocortical

soindole (BPHI) which is competitive to FPP and the availability of a potent CaaX-competitive GGTase-1 inhibitor (GGTI-298) prompted us to test the Ki-Ras alternative prenylation hypothesis.

In this article, we report the effects of BPHI FTase inhibitors on cell proliferation and on prenylation of Ras in Ki-Ras-overexpressing RTAC cells. BPHI blocked very efficiently the farnesyl transfer to Ras but not that of the geranylgeranyl moiety. However, BPHI only slightly decreased RTAC cell proliferation. Although GGTI-298 alone inhibited cell growth, we found that suppression of mitogen-activated protein (MAP) kinase activation and arrest of the cell cycle in the G<sub>0</sub>/G<sub>1</sub> phase requires both GGTI-298 and BPHI. Thus, these results suggest that a potential strategy to block efficiently Ki-Ras-dependent tumorigenic cell growth is to use the combined action of a peptidomimetic GGTase-1 inhibitor and a FPP-competitive FTase inhibitor.

## 2. Materials and methods

### 2.1. FTase, GGTase-1 and 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors

The BPHI derivative dextrogyre enantiomers RPR130401 and RPR115135 (Rhône-Poulenc Rorer, Vitry-sur-Seine, France) were synthesized and enantiomers were resolved through high performance liquid chromatography on chiral phase as described [11]. The synthesis of FTase-specific (FTI-277) and GGTase-1-specific (GGTI-298) peptidomimetics was described previously [5,7,12–14]. HMG-CoA reductase inhibitors lovastatin and simvastatin were kindly provided by Merck Sharp and Dohme Research Laboratories (Rahway, NJ, USA).

### 2.2. Cell lines and cell culture

The RTAC cells, producing Ha-Ras-V12 and overexpressing Ki-Ras, were obtained from newborn rat adrenals by transfection with a mutated Ha-ras<sup>EJ</sup> as described previously [15]. Y1 mice adrenal tumor cells, overexpressing Ki-Ras, and T24 human bladder tumor cells (expressing Ha-Ras-V12) were purchased from the American Tissue Collection (Rockville, MD, USA). Cells were grown in William's E and Ham's F10 medium (1:1, v/v) supplemented with penicillin (200 U/ml), streptomycin (200 µg/ml), 2.5% fetal bovine serum and 2.5% newborn calf serum (serum-supplemented medium (SSM)). All cell lines were propagated in a humidified 5% CO<sub>2</sub> incubator at 37°C.

### 2.3. Determination of cell proliferation by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method

Cells were seeded into 96 well plates (8000/well) and were treated daily with increasing concentrations of inhibitors (0–20 µM) or vehicle in SSM medium. At each day, one plate was taken out and the medium was replaced with 100 µl MTT (0.5 mg/ml in RPMI 1640 medium without phenol red). After 4 h incubation at 37°C, medium was discarded, MTT reaction products were dissolved in 100 µl DMSO and the absorbance was measured at 490 nm with a Micro-ELISA Auto Reader spectrophotometer (Dynatech, Alexandria, VA, USA).

### 2.4. Specific labelling and detection of isoprenylated Ras proteins

RTAC cells were plated on 60 mm Petri dishes and were treated at confluence (5 × 10<sup>6</sup> cells) as described previously [8,16]. Two consecutive treatments were carried out with 10 µM FTase inhibitors for 24 h followed by a second with 10 µM lovastatin. The cells were then incubated in 2 ml culture medium with 30 µCi/ml [<sup>3</sup>H]FPP or [<sup>3</sup>H]geranylgeranyl-pyrophosphate ([<sup>3</sup>H]GGPP) (17 Ci/mmol, Amersham, UK) for 8 h. Cells were disrupted in 1 ml lysis buffer (phosphate-buffered saline containing 1% (v/v) Triton X-100, 0.1% (w/v) sodium dodecyl sulfate (SDS), 0.5% (w/v) deoxycholic acid, 1 mM phenylmethylsulfonylfluoride, 10 µg/ml aprotinin, 10 µg/ml leupeptin and 10 µg/ml pepstatin, pH 7.4). The cell debris were removed by centrifugation at 3500 × g, 4°C for 10 min and supernatants were used for prenylated Ras analysis. Cell lysates were immunoprecipitated

with pan-Ras (Ab-3) monoclonal antibody (Oncogene Science, Uniondale, NY, USA) as described before [8]. Bound proteins were washed four times with lysis buffer and released by boiling in sample buffer. Released proteins were separated on a 14% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrotransferred to Immobilon-P membranes (Millipore, Bedford, MA, USA). Membranes were exposed on a phosphorimager (Beckman, Palo Alto, CA, USA) tritium-specific screen for 25 days.

### 2.5. Immunodetection of Ras proteins

Cells were disrupted in lysis buffer. Cellular proteins (RTAC or Y1, 5 µg; T24, 20 µg) were separated on 14% SDS-PAGE, transferred to Immobilon-P membranes and immunoblotted with pan-Ras (Ab-3) or specific anti-Ki-Ras monoclonal antibody (Ab-1; Oncogene Science). Positive reactions were visualized using peroxidase-conjugated goat anti-mouse IgG (Sigma) and an enhanced chemiluminescence (ECL) reaction system (Amersham).

### 2.6. Flow cytometry

RTAC cells were plated at 400 000 cells on 35 mm Petri dishes, in SSM, and allowed to attach overnight. Cells were treated with vehicle or inhibitors every 24 h for 48 h, harvested and 10<sup>6</sup> nuclei were prepared and stained with propidium iodide as described [17]. Aliquots containing 10<sup>4</sup> nuclei were run in a FACstar flow cytometer (Becton-Dickinson). The proportions of cells in G<sub>0</sub>/G<sub>1</sub>, S and G<sub>2</sub>/M were calculated using the CellFIT Cell-Cycle Analysis software (Ver. 2.0, Becton-Dickinson).

### 2.7. Activated MAP kinases immunoblotting

Cultured RTAC cells were treated as described under Section 2.5 and were lysed in lysis buffer with 2 mM EGTA, 2 mM EDTA and 500 µM sodium orthovanadate. Equal amounts of total proteins (100 µg) were separated on 10% SDS-PAGE and transferred to a PVDF membrane. As loading control, constitutive Ras p21 was detected in parallel for each sample with pan-Ras (Ab-3) antibody. For the detection of the phosphorylated MAP kinases, we used the anti-

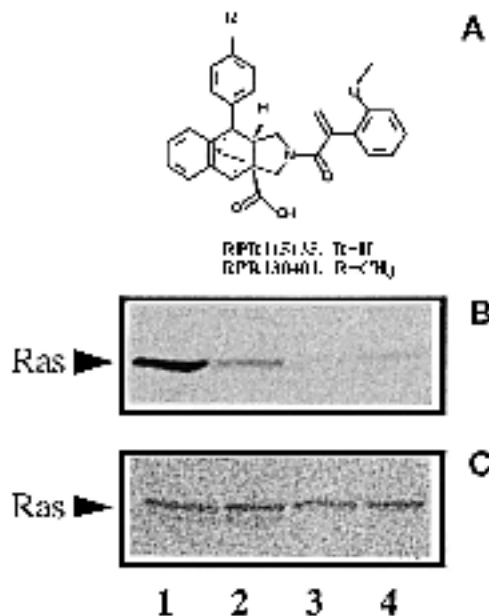


Fig. 1. BPHI FTase inhibitors and FTI-277 inhibit farnesyl but not geranylgeranyl transfer to Ras in RTAC cells. (A) The molecular structure of BPHI FTase inhibitors: RPR130401 and RPR115135. (B and C) Cells were incubated with FTase inhibitors (10 µM) for 24 h, with lovastatin and FTase inhibitors (10 µM) for another 24 h and with 30 µCi/ml [<sup>3</sup>H]FPP (B) or [<sup>3</sup>H]GGPP (C) for 8 h. Control vehicle (lane 1), FTI-277 (lane 2), RPR130401 (lane 3) and RPR115135 (lane 4). After immunoprecipitation, Ras proteins were separated by SDS-PAGE and detected by autoradiography using a tritium screen and a phosphorimager. Data are representatives of three independent experiments.

ACTIVE MAP kinase polyclonal antibody (25 ng/ml) raised against the dually phosphorylated Thr/Glu/Tyr region (pT<sup>183</sup>EpY) within the catalytic core of the active form of MAP kinase enzymes (Promega, Madison, WI, USA). A secondary peroxidase-conjugated donkey anti-rabbit IgG at 1:10 000 dilution (Promega) and an ECL system were also used. In a separate experiment, we tested the specificity of the activated MAP kinases detection, using serum-stimulated and non-stimulated RTAC cells grown in serum-free medium. A polyclonal antibody raised against total MAP kinase ERK 1 and detecting also ERK 2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) gave a similar MAP kinase detection in both samples. However, the anti-ACTIVE MAP kinase antibody leads to a signal only in the serum-stimulated sample.

### 3. Results and discussion

#### 3.1. BPHI FTase inhibitors block efficiently the farnesyl but not geranylgeranyl transfer to Ras in RTAC cells

The inhibitory effect of the novel BPHI FTase inhibitors RPR130401 and RPR115135 (Fig. 1A) and the CaaX peptidomimetic FTI-277 on farnesyl and geranylgeranyl transfer to Ras was evaluated in whole cells by blocking endogenous prenyl synthesis with lovastatin and providing [<sup>3</sup>H]FPP or [<sup>3</sup>H]GGPP in cell culture medium. RPR130401 displayed the most potent inhibitory effect on farnesyl transfer to Ras (90% at 1  $\mu$ M and 95% at 10  $\mu$ M). The other inhibitors, RPR115135 and FTI-277 at 10  $\mu$ M, showed also a clear inhibitory effect of 81 and 60%, respectively (Fig. 1B). A labelling experiment with [<sup>3</sup>H]GGPP showed that Ras proteins are geranylgeranylated in RTAC cells (Fig. 1C, lane 1). None of the FTase inhibitors decreased the geranylgeranylation of Ras

(Fig. 1C). Consequently, BPHI and peptidomimetic FTase inhibitors were highly specific to FTase over GGTase-1 *in vivo*. Thus, the geranylgeranyl transfer to Ras did not proceed through FTase in whole cells.

#### 3.2. RTAC cell proliferation is resistant to FPP-competitive and peptidomimetic FTase inhibitors but is sensitive to lovastatin and simvastatin

We next determined whether, at concentrations of FTI-277 and RPR130401 that are highly selective for FTase over GGTase-1, these agents are capable of inhibiting proliferation. Fig. 2 shows that FTI-277 (10  $\mu$ M) and RPR130401 (10  $\mu$ M) inhibit RTAC cells proliferation by only 10 and 30%, respectively. The IC<sub>50</sub> for RPR130401 is 30  $\mu$ M. Similarly, Fig. 2A,B shows that Y1 cells were not sensitive to FTI-277 and RPR130401. Conversely, T24 cells were sensitive to both FTase inhibitors in the 1–20  $\mu$ M range. RPR130401 was the most efficient FTase inhibitor of proliferation of partly resistant cells (RTAC and Y1 cells) but FTI-277 (1–10  $\mu$ M) was equally or even more efficient (at 20  $\mu$ M) than RPR130401 on T24 sensitive cells. Ras analysis by SDS-PAGE (Fig. 2D) showed abundant Ki-Ras protein in RTAC and Y1 cells while Ki-Ras was absent in T24 cells which express Ha-Ras-V12 [18,19]. These results suggest that the lack of sensitivity of RTAC and Y1 cells is due to resistance of Ki-RasB prenylation to FTase inhibitors.

Furthermore, RTAC cells were very sensitive to the HMG-CoA inhibitors lovastatin (IC<sub>50</sub> = 7  $\mu$ M) and simvastatin (IC<sub>50</sub> = 2  $\mu$ M, Fig. 2C). All together, these results suggest

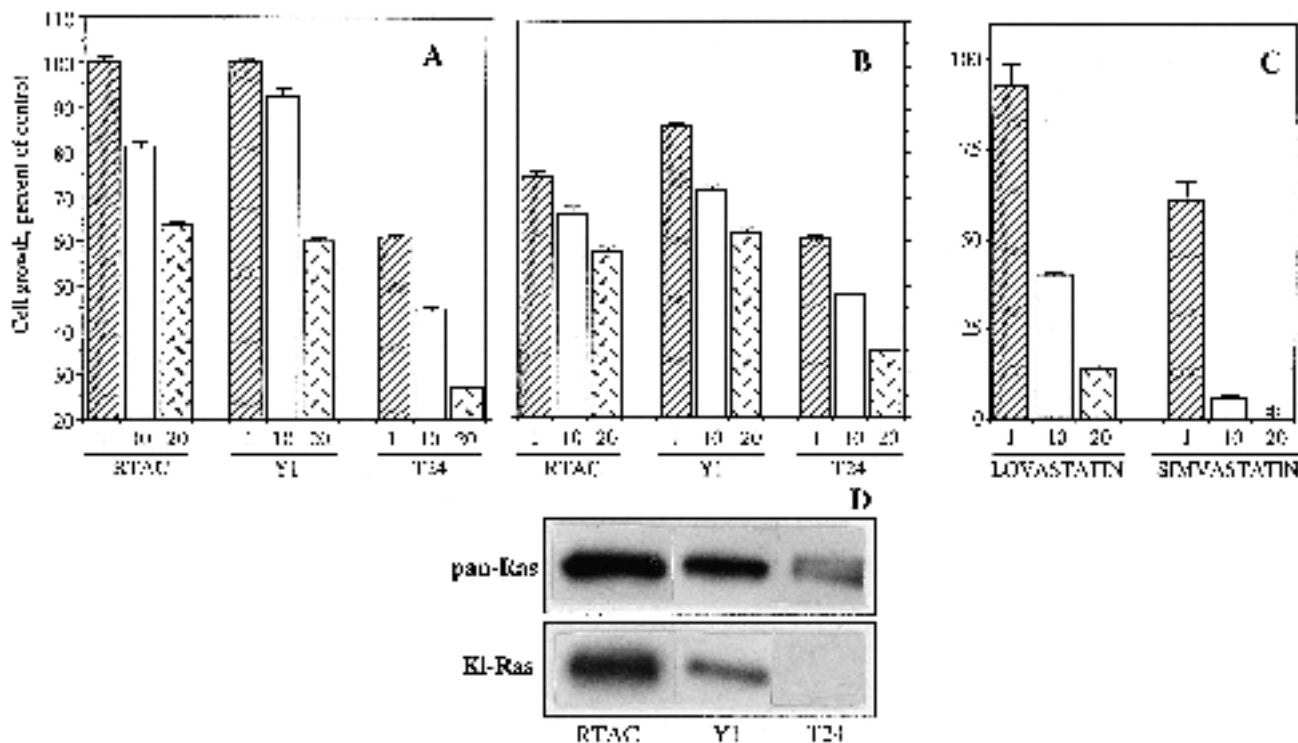


Fig. 2. Proliferation of RTAC cells is partially resistant to FTase inhibitors but sensitive to HMG-CoA reductase inhibitors. (A and B) Partial inhibition of cell proliferation by FTI-277 (A) and RPR130401 (B) (1, 10 and 20  $\mu$ M) in RTAC and Y1 cells as compared to strong inhibition in T24 cell lines. (C) Effect of lovastatin and simvastatin (1, 10 and 20  $\mu$ M) on cell proliferation in RTAC cells. (\*) Partial cell death observed. Cells grown in 5% SSM were treated each day for 4 days with fresh medium and indicated concentrations of inhibitors. Cell numbers were measured in octuplicate in 96 well plates as described under Section 2 and shown at day 4. (D) Immunodetection of p21 Ras proteins in RTAC, Y1 and T24 cells with pan-Ras (Ab-3) and Ki-Ras antibodies. Data are representatives of three independent experiments.

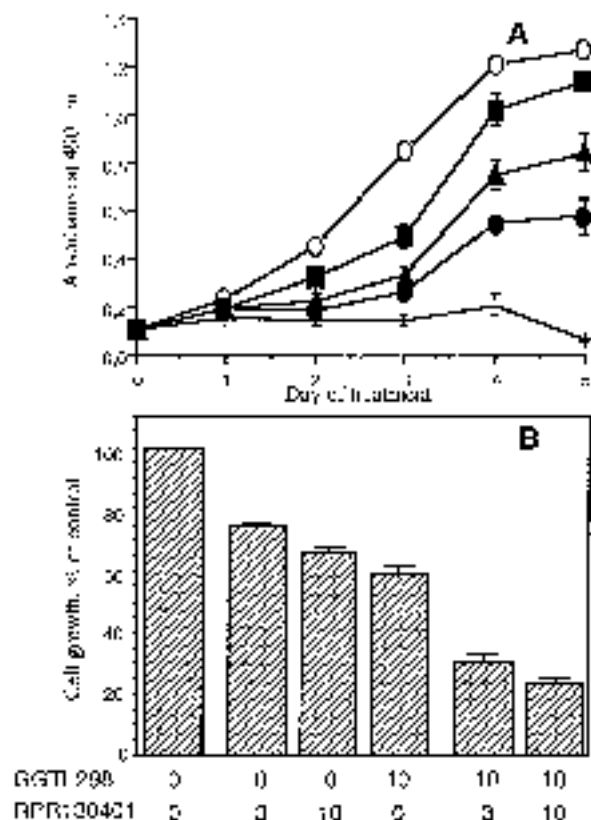


Fig. 3. GGTI-298 alone (A) and combination of GGTI-298 and RPR130401 (B) inhibit RTAC cell proliferation. (A) RTAC cells were treated daily with the vehicle (○) or with GGTI-298 at 3 (■), 10 (▲), 12 (●) and 15 (+) μM. (B) Cells were treated daily with vehicle alone, inhibitors alone or in combination at the given concentrations (in μM). Cell numbers were measured each day in 96 well plates with the MTT colorimetric method and shown for each day (A) or at day 4 (B). Data are the mean of octuplicate measurements and the data are representatives of three independent experiments.

that an alternative prenylation to that catalyzed by FTase may be involved in RTAC cell proliferation.

### 3.3. RTAC cell proliferation is blocked by GGTI-298 at high concentrations and by the combined treatment with FTase inhibitors and GGTI-298 at low, non-cytotoxic concentrations

GGTI-298 (3–15 μM) inhibited RTAC cell proliferation in a dose-dependent manner with an  $IC_{50}$  of 11 μM (Fig. 3A). A slight cytostatic effect (17%) was shown at 3 μM and a more marked cytostatic effect was observed at 10 μM (41%). Cells treated with GGTI-298 (15 μM) for 4 days remained quiescent. However, on day 5, severe cell lysis occurred. GGTI-298 (10 μM) for up to 5 days showed no cytotoxicity. When combined, GGTI-298 (10 μM) and RPR130401 (3 or 10 μM) inhibited cell proliferation by 70 and 77%, respectively (Fig. 3B). Each inhibitor alone inhibited proliferation only by 35–40% (Fig. 3B). Thus, both farnesylated and geranylgeranylated proteins are involved in RTAC cell proliferation.

### 3.4. FTase inhibitor RPR130401 and GGTI-298 co-treatment is required for cell cycle arrest in the $G_0/G_1$ phase

Table 1 shows that co-treatment with RPR130401 and GGTI-298, but not mono-treatment, resulted in an increase of the percentage of cells in the  $G_0/G_1$  phase of the cell cycle from 58 to 76%. This value is similar to that obtained with lovastatin at 20 μM (79%). Thus, lovastatin alone and a combination of GGTI-298 and RPR130401 were able to arrest cells in  $G_0/G_1$ . Furthermore, a marked amount of subdiploid cells (16%) was also observed with GGTI-298 (15 μM) and lovastatin (13% at 20 μM). This observation is in favor of an apoptotic effect of GGTI-298 at concentrations higher than 10 μM and is consistent with the cell lysis observed after a 4 day treatment with 15 μM GGTI-298 (Fig. 3A).

### 3.5. Co-treatment of RPR130401 and GGTI-298 blocks MAP kinase activation in RTAC cells

MAP kinases ERK 1 and ERK 2 were activated in RTAC cells maintained in SSM, as measured by Western blotting using an antibody specific for dually phosphorylated (pT<sup>183</sup>EpY) MAP kinases. This activation was blocked by lovastatin (not shown) but not by RPR130401, FTI-277 or GGTI-298 alone (Fig. 4). In contrast, a co-treatment with

Table 1  
Effects of GGTase-1 and FTase inhibitors on cell cycle phase distribution in RTAC cells

Treatment	μM	$G_0/G_1$ (%)	$G_2/M$ (%)	Sub $G_1$ (%)
Control	<sup>a</sup>	58	30	1
Lovastatin	20	79	16	13
GGTI-298	3	59	28	4
	10	62	26	5
	15	62	27	16
FTI-277	3	57	29	3
	10	60	30	4
RPR130401	3	58	30	1
	10	66	30	2
GGTI-298/FTI-277	10/3	60	29	4
	10/10	68	26	7
GGTI-298/RPR130401	10/3	75	23	3
	10/10	76	21	4

RTAC cells were treated with either vehicle, inhibitors alone or a combination of GGTase-1 and FTase inhibitors each day for 2 days. Cell cycle distribution was determined on  $10^4$  cells by DNA propidium iodide staining and flow cytometry measurement as described under Section 2. Data are representatives of three separate experiments.

<sup>a</sup>Drug vehicle only.

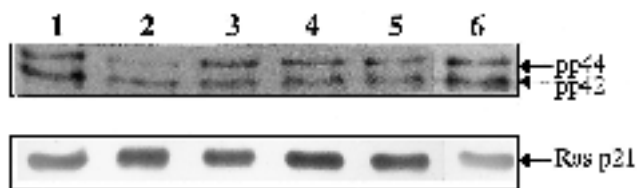


Fig. 4. Co-treatment with GGTI-298 and RPR130401 suppresses activation of MAP kinase signaling in RTAC cells. RTAC cells were treated every 24 h for 48 h with vehicle (lane 1), 10  $\mu$ M GGTI-298 and 10  $\mu$ M RPR130401 (lane 2), 10  $\mu$ M GGTI-298 and 10  $\mu$ M FTI-277 (lane 3), 10  $\mu$ M RPR130401 (lane 4), 10  $\mu$ M FTI-277 (lane 5) and 10  $\mu$ M GGTI-298 (lane 6). Cells were lysed and equivalent amounts of proteins (100  $\mu$ g) were separated on SDS-PAGE and immunoblotted with anti-ACTIVE MAPK antibody recognizing both dually phosphorylated (pTepY) forms of MAP kinases (pp42, pp44) as described under Section 2. Constitutive Ras p21 detection was carried out with pan-Ras (Ab-3) antibody as a loading control.

RPR130401 and GGTI-298 suppressed the MAP kinase activation. Thus, this shows that both a FTase inhibitor (BPHI) and GGTase-1 inhibitor (GGTI-298) were required for disrupting Ki-Ras protein signaling.

Our results clearly demonstrate that BPHI FTase inhibitors are potent and selective at blocking farnesyl but not geranylgeranyl transfer to Ras in RTAC cells. This is consistent with our *in vitro* data where BPHI resulted in inhibition of the farnesylation of Ki-Ras in the nM range in a highly selective manner (100-fold for FTase over GGTase-1) [11]. FTI-277 was also shown to be specific for FTase (0.2  $\mu$ M  $IC_{50}$  for Ha-Ras processing) over GGTase-1 (40  $\mu$ M  $IC_{50}$  for Rap1a processing) [5]. Furthermore, our results are consistent with findings that Ki-Ras is geranylgeranylated by GGTase-1 in cultured cells [20–22] and not by FTase. This occurs despite the fact that this enzyme was able to transfer *in vitro* both farnesyl and geranylgeranyl moieties from FPP and GGPP to Ki-Ras proteins but with a higher  $K_m$  for the latter [23–25].

Resistance of Ki-Ras-expressing RTAC cells to cell growth inhibition by CaaX peptidomimetics and FPP-competitive inhibitors at concentrations that inhibit FTase but not GGTase-1 suggests that Ras is geranylgeranylated and is capable of inducing the activation of the mitogenic transduction cascade downstream to MAP kinase which contributes to continued proliferation. Geranylgeranylated Ras appears to be as efficient as farnesylated Ras at activating MAP kinases, as evident by the fact that MAP kinases continue to be phosphorylated even in the presence of FTase inhibitors. Similarly, when GGTase-1 was blocked by GGTI-298 at 10  $\mu$ M, Ras was still farnesylated by FTase and MAP kinases were activated. At a higher concentration (15  $\mu$ M), GGTI-298 inhibited cell proliferation. This is likely due to the inhibition of geranylgeranylated proteins such as RhoA and Rac1 that are involved in the cell cycle control and possibly apoptosis [26–28]. More importantly, when both Ras farnesylation and its alternative geranylgeranylation were blocked by a BPHI FTase inhibitor and the CaaX peptidomimetic GGTI-298 at not really cytotoxic and non-apoptotic concentrations, activation of MAP kinases was suppressed, the cell cycle was blocked in the  $G_0/G_1$  phase and cell proliferation was considerably decreased.

Two binding sites, one for the isoprenoid and the other for the CaaX peptide substrates, have been identified on the  $\beta$  subunit of FTase by X-ray crystallography [29] and postulated

to reside on the related subunit of GGTase-1. The efficiency of the BPHI and GGTI-298 combination used in this study may be explained by the absence of competition between the two inhibitors since they bind two different sites on the respective FTase and GGTase-1 enzymes. All together, these results suggest that an efficient strategy to block tumoral proliferation of Ki-Ras-expressing cells is to combine FTase and GGTase-1 inhibitors acting on two types of sites of the two  $\beta$  subunits: one binding to the isoprenoid site of the FTase  $\beta$  subunit (FPP-competitive) and the second one binding to the protein binding site of GGTase-1  $\beta$  subunit (CaaX peptidomimetic).

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