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J-104,871, a Novel Farnesyltransferase Inhibitor, Blocks Ras Farnesylation *In Vivo* in a Farnesyl Pyrophosphate-Competitive Manner

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ABSTRACT

Farnesylation of the activated *ras* oncogene product by protein farnesyltransferase (FTase) is a critical step for its oncogenic function. Because squalene synthase and FTase recruit farnesyl pyrophosphate as a common substrate, we modified squalene synthase (SS) inhibitors to develop FTase inhibitors. Among the compounds tested, a novel FTase inhibitor termed J-104,871 inhibited rat brain FTase with an IC_{50} of 3.9 nM in the presence of 0.6 μM farnesyl pyrophosphate (FPP), whereas it scarcely inhibited rat brain protein geranylgeranyltransferase-I or SS. The *in vitro* inhibition of rat brain FTase by J-104,871 depends on the FPP concentration but not on the concentration of Ras peptide. Thus, *in vitro* studies strongly suggest that J-series compounds have an FPP-competitive nature. J-104,871 also inhibited Ras processing in activated H-ras-transformed NIH3T3 cells with an IC_{50} value of 3.1 μM. We tested the effects of lovastatin and zaragozic acid A, which modify cellular FPP levels, on Ras processing of J-104,871. Lovastatin, a hepatic hydroxymenthyl coenzyme A reductase inhibitor that reduced the cellular FPP pool, increased the activity of J-104,871, whereas 3 μM zaragozic acid A, an SS inhibitor that raised the FPP level, completely abrogated the activity of J-104,871 even at 100 μM. These results suggest that J-104,871 inhibits FTase in an FPP-competitive manner in whole cells as well as in the *in vitro* system. Furthermore, J-104,871 suppressed tumor growth in nude mice transplanted with activated H-ras-transformed NIH3T3 cells.

Ras plays a crucial role in cellular signal transduction pathways (Barbacid, 1987; Lowy, 1993). Similar to other low-molecular-weight GTP-binding proteins, Ras protein exists in two states: a GTP-bound active state and a GDP-bound inactive state. Normal Ras possesses GTPase activity, which leads to the hydrolysis of bound GTP to GDP, resulting in termination of the mitogenic signal. Point mutations in the *ras* oncogenes that lock Ras into its active GTP-bound state cause malignant transformations (Gibbs *et al.*, 1984; Bourne *et al.*, 1991; Scheffzek *et al.*, 1997). Such oncogenically mutated forms of Ras are found in a wide variety of human tumors, most notably in 90% of pancreatic adenocarcinomas and 50% of colon cancers (Bos, 1989; Barbacid, 1990; Rodenhuis, 1992). Ras protein must be localized to the plasma membrane to transform cells. This localization is achieved by post-translational modifications directed by the Ras protein carboxyl-terminal CAAX sequence, where C is cysteine, A is an aliphatic residue, and X is preferably serine or methionine (Willumsen *et al.*, 1984; Hancock *et al.*, 1989; Schafer *et al.*, 1989). The first and most critical modification is farnesylation of the conserved cysteine, catalyzed by the FTase (Reiss *et al.*, 1990; Kato *et al.*, 1992). Subsequently, the sequence AAX is proteolytically cleaved, and the newly formed carboxyl-terminal farnesyl cysteine is finally methylated (Zhang and Casey, 1996).

Consequently, inhibitors of FTase have been proposed as potential agents for treating cancers in which Ras plays a pivotal role (Gibbs, 1991). Synthetic FTase inhibitors have been designed based on the structures of two substrates that are involved in the reaction, FPP and Ras CAAX tetrapeptide. Ras-competitive inhibitors that have been synthesized, both CAAX-related and CAAX-unrelated, display nanomolar inhibitory potency toward FTase but retain selectivity against GGTase-I; some of these inhibitors have been shown to inhibit the growth of Ras-dependent tumors in nude mice (James *et al.*, 1993; Kohl *et al.*, 1994; Bishop *et al.*, 1995; Nagasu *et al.*, 1995; Sun *et al.*, 1995). However, with the exception of FTase inhibitors reported by McNamara *et al.*

ABBREVIATIONS: FTase, protein farnesyltransferase; FPP, farnesyl pyrophosphate; GGTase, protein geranylgeranyltransferase; SS, squalene synthase; GGPP, geranylgeranyl pyrophosphate; DMEM, Dulbecco's modified Eagle's medium; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; MTT, 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HMG-CoA, hepatic hydroxymethylglutaryl coenzyme A.

(1997), none of the synthetic FPP-competitive inhibitors has been shown to exhibit antitumor activity *in vivo* as well as *in vitro*. We have previously reported novel SS inhibitors (Iwasawa *et al.*, 1995, 1996). Because SS and FTase recruit FPP as a common substrate, we modified our SS inhibitors to develop FTase inhibitors. In this study, we demonstrate that a novel J-series compound termed J-104,871 inhibits FTase potently and selectively in an FPP-competitive manner. *In vitro* and *in vivo* analyses revealed that J-104,871 is potentially useful in deciphering the biochemical mechanism of Ras prenylation. Furthermore, this compound has therapeutic potential in Ras-related oncogenesis.

Experimental Procedures

Materials. All-*trans* [^{3}H]FPP and All-*trans* [^{3}H]GGPP were purchased from Dupont-New England Nuclear (Boston, MA). Lovastatin, simvastatin, and zaragozic acid A were provided by Merck Research Labs (West Point, PA). J-104,871 [(4*R**,5*S**)-5-{*N*-[(1*R*,2*R*,4*E*)-5-(2-benzoxazolyl)-1-methyl-2-(3,4-methylenedioxyphenyl)-4-pentenyl]-*N*-(2-naphthylmethyl)carbamoyl}-1,3-dioxolane-2,2,4-tricarboxylic acid] (Fig. 1) and NB-598 were synthesized in our laboratory. Anti-H-Ras antibody (NCC-RAS-004) was purchased from Nihonkayaku (Tokyo, Japan) and anti-Rap1A antibody (sc-311) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, California). NIH3T3 cells with stable expression of activated H-Ras (Gln61Leu) were kindly provided by Dr. T. Sekiya (National Cancer Center Research Institute, Tokyo, Japan).

***In vitro* enzyme assay for FTase, GGTase-I, and SS.** FTase and GGTase-I were partially purified from rat brain by ammonium sulfate fractionation and Mono Q column chromatography as described by Reiss *et al.* (1990). Biotinylated KTSCVIM (peptide Lys-Thr-Ser-Cys-Val-Ile-Met) as a peptide substrate of FTase and biotinylated NPFREKKFFCAIL (peptide Asn-Pro-Phe-Arg-Glu-Lys-Lys-Pro-Pro-Cys-Ala-Ile-Leu) as a substrate of GGTase-I were synthesized by a peptide synthesizer (Model 431A; Applied Biosystems, Foster City, CA). FTase assay was performed according to the method described previously by Reiss *et al.* (1991). Briefly, the standard reaction mixture (25 μl total) contained 50 mM Tris·HCl, pH 7.5, 20 mM KCl, 5 mM $MgCl_2$, 0.2% (v/v) *n*-octyl-β-D-glucopyranoside, 1 mM dithiothreitol, 0.6 μM [^{3}H]FPP, 3.6 μM biotinylated KTSCVIM, partially purified FTase, and the indicated concentrations of compounds or dimethyl sulfoxide as vehicle control (2% v/v, final). Reactions were started by adding the enzyme and stopped after 20 min of incubation with 100 μl of stop reagent containing streptavidin-linked scintillation proximity assay beads (Amersham, Tokyo, Japan). FTase activity was determined by measuring the incorporation of the [^{3}H]farnesyl group from [^{3}H]FPP into the substrate peptide. Radioactivity was counted using a liquid scintillation counter (TRI-CARB 2300TA; Packard, Meriden, CT). GGTase-I assay was carried out in a similar manner except that 0.6 μM [^{3}H]GGPP, 3.6 μM biotinylated NPFREKKFFCAIL, and partially purified GGTase-I were used.

SS activity was determined as described by Bergstrom *et al.* (1993) by using microsomes prepared from Hep G2 cells pretreated for 24 hr with 1 μM simvastatin, an HMG-CoA reductase inhibitor, for induction of this enzyme protein. Briefly, 40 μl of assay mixture included 100 mM potassium phosphate buffer, pH 7.5, 5 mM $MgCl_2$, 10 mM dithiothreitol, 2 mM NADPH, 10 μM [^{3}H]FPP, and 1.4 μg of protein. Assays were run for 20 min at 37° in the presence of 0.5 μM NB598, a squalene epoxidase inhibitor, to avoid further processing of squalene by squalene epoxidase that co-exists in the microsomal preparation (Horie *et al.*, 1990). The enzyme reaction was terminated with 6.5 μl of 0.75 M EDTA and 3.5 μl of unlabeled 0.5% squalene. Twenty microliters of each sample was spotted on a silica gel G plate (Art F 254; Merck, Darmstadt, Germany), dried, and washed twice with 1% SDS/0.2 mM Tris·HCl, pH 7.5. By this procedure, [^{3}H]FPP was extracted into the SDS/Tris solution, but [^{3}H]squalene was left on the silica gel plate. The squalene spot was scraped and the radioactivity was counted by a liquid scintillation counter.

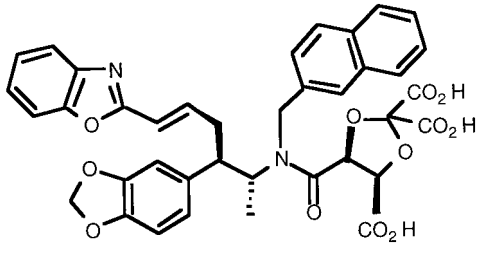

Fig. 1. Structure of J-104,871. J-104,871 is a representative compound.

H-Ras and Rap processing assay in cells. An H-Ras processing assay was performed as described previously by Garcia et al. (1993). On day 0, activated H-*ras*-transformed NIH3T3 cells were seeded in DMEM containing 10% calf serum in six-well tissue culture dishes. On day 2, the medium was changed to DMEM containing 2% calf serum, and the test compounds were added. Another 24 hr later (day 3), the cells were harvested and lysed in lysis buffer (1% Nonidet P40, 20 mM HEPES, 5 mM $MgCl_2$, 10 μg/ml of aprotinin, 2 μg/ml of leupeptin, 2 μg/ml of antipain, 0.5 mM phenylmethylsulfonyl fluoride). The lysate was separated by centrifugation and the supernatant was used as a cell extract. Proteins (10 μg) of each cell extract were separated by SDS-PAGE in 12% acrylamide gels. Proteins blotted onto a nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany) were probed with a monoclonal anti-H-Ras antibody. All blots were developed using enhanced chemiluminescence reagents (Amersham). Densitometric analysis of the bands corresponding to farnesylated and nonfarnesylated Ras protein in each lane was performed to determine the percent inhibition of protein farnesylation. A Rap-processing assay was performed using a method similar to that of James *et al.* (1996). Cells were cultured, harvested, and lysed in the lysis buffer as described above, except that Nonidet P40 was omitted. The lysate was separated by centrifugation at $10^5 \times g$ for 30 min. The supernatant (S-100) was transferred to a new tube, and the remaining pellet ($10^5 \times g$ pellet) was resuspended in the lysis buffer used in the Ras-processing assay. Proteins recovered in the S-100 fraction (1.5 μg) and in the solubilized $10^5 \times g$ pellet fraction (5 μg) were resolved by SDS-PAGE as described above. The Rap protein in each fraction was detected with rabbit polyclonal anti-Rap1A antibody. Protein concentrations were determined using

TABLE 1

Dominant selectivity of J-104,871 for FTase

Enzyme assays were performed as described in Experimental Procedures. Each value is the mean ± standard deviation in three separate determinations.

Compound	IC_{50}		
	FTase	GGTase-I	SS
		nM	
J-104,871	3.9 ± 0.9	1,300 ± 200	>100,000

TABLE 2

Competition of J-104,871 for FPP

Mutual sets of two distinct concentrations of FPP and Ras CAAX (biotinylated KTSCVIM) were designed. The IC_{50} values of J-104,871 were determined as described in Experimental Procedures.

Concentration		IC_{50}
FPP	Ras CAAX	
μM		*nM*
0.6	3.6	4.8
6	3.6	48
0.6	0.36	6.8

the Bradford method with commercial dye preparation (Bio Rad, Hercules, CA).

Cell morphology.. On day 0, activated H-*ras*-transformed NIH3T3 cells and untransformed NIH3T3 cells were seeded in DMEM containing 10% calf serum in six-well tissue culture dishes. On days 2 and 5, the medium was changed to fresh medium containing the test compounds. On day 6, the cells were microscopically monitored for morphological changes.

Colony formation assay.. 5×10^3 cells of H-*ras*-transformed NIH3T3 were seeded on 24-well tissue culture dishes in 0.4 ml of 0.28% Noble agar (Difco, Detroit, MI) in DMEM containing 10% calf serum over 0.5 ml of 0.56% Noble agar in the same culture medium. After 14 days, 0.2 ml of 0.5 mg/ml MTT in water was added and the agar was incubated for overnight. The number of stained colonies was analyzed with a colony counter (PCA-11; System Science, Tokyo, Japan).

***In vivo* xenograft assays and Ras processing assay.** On day 0, activated H-*ras*-transformed NIH3T3 cells (10^5 cells/mouse) were injected subcutaneously into the right flank of female nude mice (8 weeks old). On the subsequent 6 days, mice were dosed with test compound intraperitoneally once daily ($n = 5$). Control animals ($n = 5$) received saline vehicle on the same schedule. On days 4 and 7, tumor volume was calculated according to the following equation: tumor volume (mm^3) = (Length $\times$ width^2) / 2. Statistical significance between the control and treated groups was evaluated using Student's t test. On day 7 (24 hr after the last dose), the tumor was excised, lysed, and immunoblotted with anti-H-ras antibody as described above.

Results

J-104,871 is a potent and selective FTase inhibitor. Because FPP is a common substrate for both FTase and SS, we suspected that our SS inhibitors (Iwasawa *et al.*, 1995, 1996) could serve as FTase inhibitors as well. Among the SS inhibitors we tested, J-104,133 was found to be a potential lead compound for developing FTase inhibitors (Aoyama *et al.*, 1998). Through modification and optimization, we developed the potent and selective FTase inhibitor, J-104,871 (Fig.

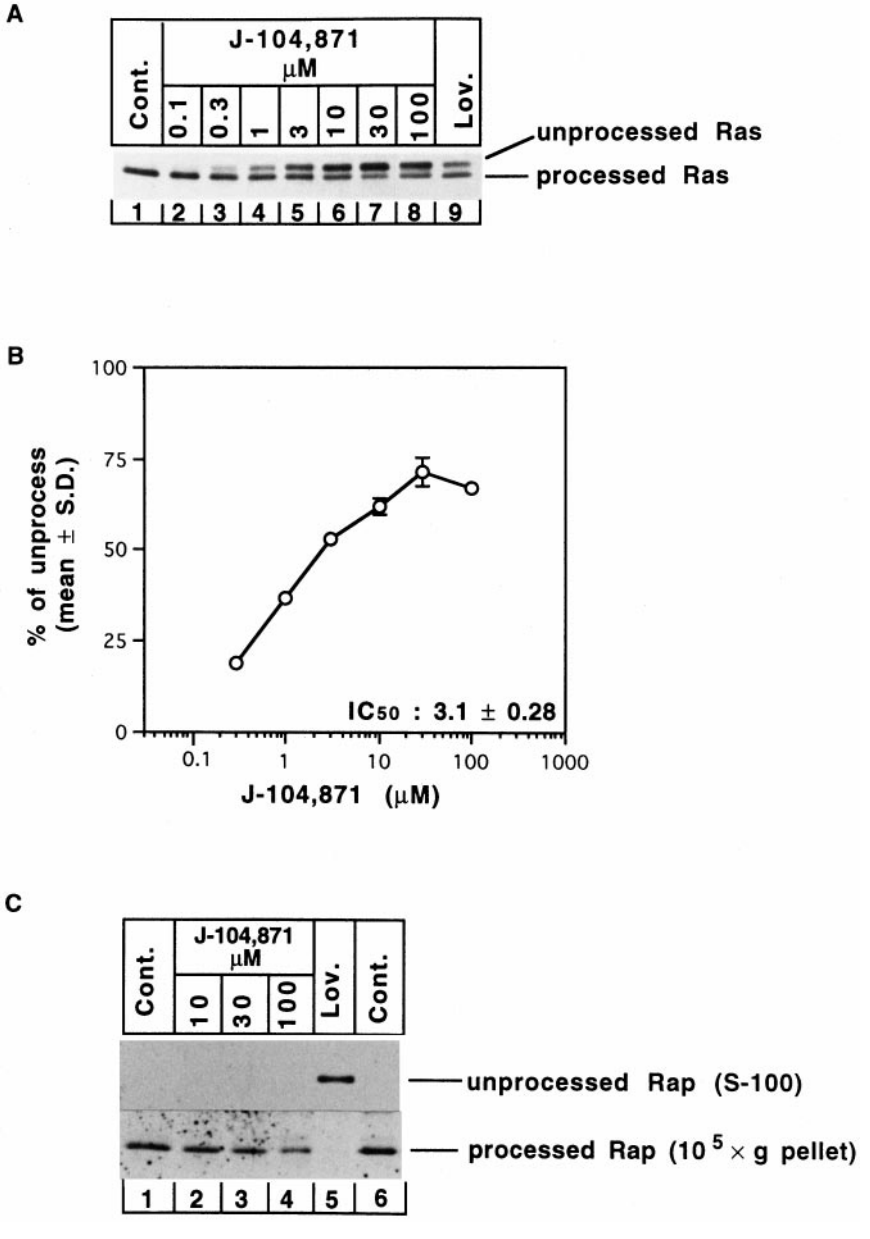

Fig. 2. J-104,871 inhibits Ras processing but not Rap processing in activated H-*ras*-transformed NIH3T3 cells. Cells were incubated in the presence of either 0.1% dimethyl sulfoxide (*Cont.*) or the indicated concentrations of J-104,871 or 50 μM of lovastatin (*Lov.*). Cells were harvested after 24 hr, lysed, and A, Ras, or C, Rap1A, was detected by immunoblotting as described in Experimental Procedures. B, A densitometric analysis of the bands corresponding to the processed and unprocessed Ras protein observed in A was performed. Percentage of unprocessed form was determined as follows: percentage of unprocessed Ras = unprocessed Ras/(unprocessed Ras + processed Ras) $\times$ 100 in each lane). The IC_{50} value is the mean $\pm$ standard deviation of three separate determinations.

1), with an IC_{50} value of 3.9 nM against FTase in the presence of 0.6 μM FPP (Table 1). The IC_{50} value of J-104,871 for GGTase-I was 1300 nM in the presence of 0.6 μM GGPP. Because the K_m values of FTase for FPP and GGTase-I for GGPP were about 10 nM (data not shown) as reported previously (Pompliano *et al.*, 1992; Fang *et al.*, 1994), J-104,871was shown to be highly selective for FTase over GGTase-I. For SS assay, 10 μM of FPP was used for the substrate. [The K_m value of SS for FPP was about 1 μM as reported previously (Bergstrom *et al.*, 1993).] Under this condition, J-104,871, with an IC_{50} value of more than 10 μM (Table 1), scarcely inhibited SS.

J-104,871 inhibits FTase with respect to FPP competitiveness. Next, we examined the inhibitory features of J-104,871. Because J-104,871 is a reversible, tight-binding inhibitor, a quantitative description could not be based on a double-reciprocal plot. Therefore, we determined the IC_{50} values of J-104,871 against distinct concentrations of FPP and biotinylated KTSCVIM. The IC_{50} value of J-104,871 rose from 4.8 nM to 48 nM as the FPP concentration increased from 0.6 μM to 6 μM, whereas it was not influenced by the concentration of peptide substrate (Table 2). These findings suggest that J-104,871 inhibits FTase activity in a competitive manner with respect to FPP but not to Ras protein.

J-104,871 blocks Ras processing but not Rap processing in H-ras-transformed NIH3T3 cells.. To examine the effects of J-104,871 on Ras processing in whole cells, we used activated H-*ras*-transformed NIH3T3 cells. Processed and unprocessed Ras protein was resolved by SDS-PAGE, followed by immunoblotting with anti-H-Ras antibody. The faster-migrating immunoreactive band represents mature, fully processed Ras, whereas the slower-migrating form is unprocessed protein (Garcia et al., 1993). The control cells contained only mature, processed Ras protein. After 24 hr of treatment with J-104,871, the dose-dependent accumulation of unprocessed Ras was observed (Fig. 2, A and B). The concentration that gave 50% unprocessed Ras was calculated as the IC_{50} value; this value of J-104,871 for Ras processing was 3.1 μM (Fig. 2B). To analyze Rap1A processing (geranylgeranylation), the cells were fractionated into cytosol (S-

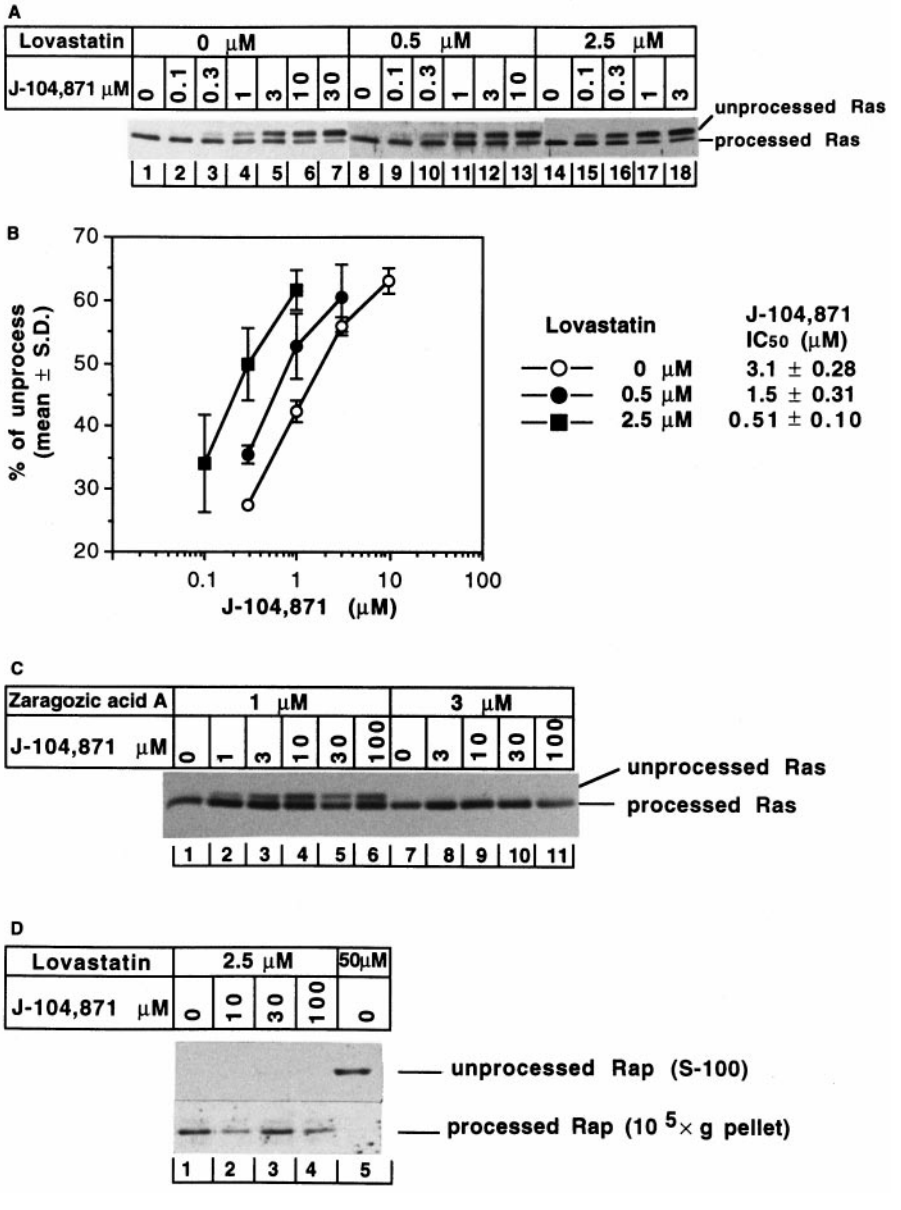

Fig. 3. J-104, 871 inhibits Ras processing depending on FPP concentration. Cells were incubated in the presence of either 0.1% dimethyl sulfoxide or the indicated concentrations of J-104, 871 A and D, plus or minus lovastatin, or C, plus zaragozic acid A. Cells were harvested after 24 hr, lysed, and in A and C, Ras, or in D, Rap1A, was detected by immunoblotting as described in Experimental Procedures. B, A densitometric analysis of bands corresponding to processed and unprocessed Ras protein observed in A was performed. Each value is the mean ± standard deviation of three separate determinations. Note that the IC_{50} value of J-104,871 varies depending on the lovastatin concentration.

100) and membrane fractions ($10^5 \times g$ pellet) and immunoblotted with anti-Rap1A antibody after SDS-PAGE. As shown in Fig. 2C, Rap1A processing was not affected by J-104,871 even at 100 μM, whereas 50 μM lovastatin, which inhibits HMG-CoA reductase, lowered the cellular levels of isoprenyl substrates (FPP and GGPP) (Schafer *et al.*, 1989), thereby hampering the activity of isoprenyl transferases (FTase and GGTase I and II), and inhibited Rap1A processing to the cell membranes with concomitant accumulation of nonprenylated Rap protein in the cytosolic fraction (Fig. 2C). Interestingly, J-104,871 up to 100 μM suppressed disordered growth and morphological change of H-ras-transformed cells with no apparent cytotoxic effects (Fig. 4A).

FPP level modulates J-104,871 activity on cellular processing of Ras. Lovastatin is known to inhibit HMG-CoA reductase and hence reduce the cellular level of FPP. This inhibitor did not block Ras processing at concentrations up to 2.5 μM (Fig. 3A, *lane 14*). It is interesting that over such a range of lovastatin concentrations (0.5 and 2.5 μM), the inhibitory effect of J-104,871 on Ras processing was potentiated (Fig. 3A). The IC_{50} values of J-104,871 were 3.1, 1.5, and 0.51 μM in the presence of 0, 0.5, and 2.5 μM lovastatin, respectively (Fig. 3B). In contrast, in the presence of zaragozic acid A, an SS inhibitor that increases the FPP pool (Bergstrom *et al.*, 1993), J-104,871 lost its activity as a Ras-processing inhibitor. In the presence of a high concentration (3 μM) of zaragozic acid A, J-104,871 even at 100 μM did not inhibit Ras processing (Fig. 3C). These results correlate well with those of the *in vitro* kinetic studies, in which we observed the FPP-competitive aspect of the J-compound. Lovastatin-mediated potentiation of the effect of J-104,871 on Rap processing was not observed even at a high concentration (100 μM) of the J-compound (Figs. 2C and 3D).

Lovastatin also potentiated the morphology-altering effect of J-104,871 in transformed NIH3T3 cells (Fig. 4, compare B with A). In contrast, zaragozic acid A abrogated this effect of J-104,871 (Fig. 4, compare C with A). These results also correlate well with the data obtained in the *in vitro* kinetic studies and a cell-level assay of Ras processing (Table 2; Fig. 3).

J-104,871 suppresses colony formation.. Next, we examined the effect of J-104,871 on the ability of activated

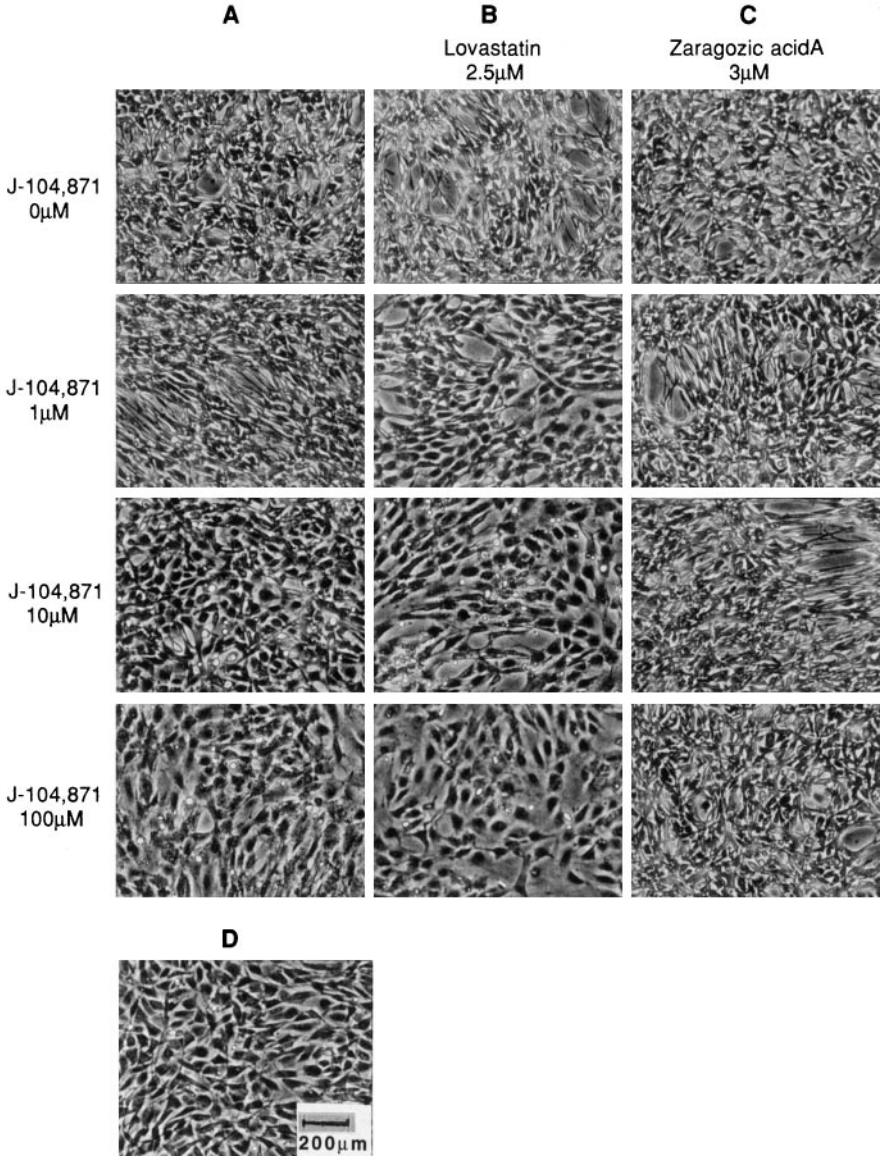

Fig. 4. J-104,871 suppresses disordered growth and morphological change of activated H-*ras*-transformed NIH3T3 cells. Cells were incubated for 4 days in the presence of 0.1% dimethyl sulfoxide or the indicated concentrations of J-104,871 (A), + lovastatin (B), + zaragozic acid A (C). D, Untransformed NIH3T3 cells. Cells were microscopically monitored and photographed under contrast at a magnification of 30×.

H-*ras*-transformed NIH3T3 cells to form colonies when grown in soft agar. This assay is especially relevant to antitumor activity because colony formation in soft agar correlates well with tumorigenicity in the nude mouse (Shin *et al.*, 1975). J-104,871 inhibited colony formation dose dependently with an IC_{50} value of 27.5 ± 1.54 μM (Fig. 5). At this concentration, J-104,871 inhibited Ras processing potently (Fig. 2, A and B) and had no effect on either geranylgeranylated protein (Fig. 2C) or nonspecific cytotoxicity (Fig. 4A).

J-104,871 suppresses tumor growth in a nude mouse xenograft model.. Finally, we examined the *in vivo* antitumor activity of J-104,871. Female nude mice were transplanted with activated H-*ras*-transformed NIH3T3 cells. Doses of J-104,871 at 40 or 80 mg/kg suppressed tumor growth by 28% and 52%, respectively (Fig. 6A). The inhibition of Ras processing in tumor tissues (Fig. 6B) correlated well with the suppression of tumor growth by this compound. Although tumors continued growing even at the higher dose (80 mg/kg), the data presented here demonstrate *in vivo* suppression of tumor growth by an FPP-competitive FTase inhibitor. We are now searching for more potent compounds.

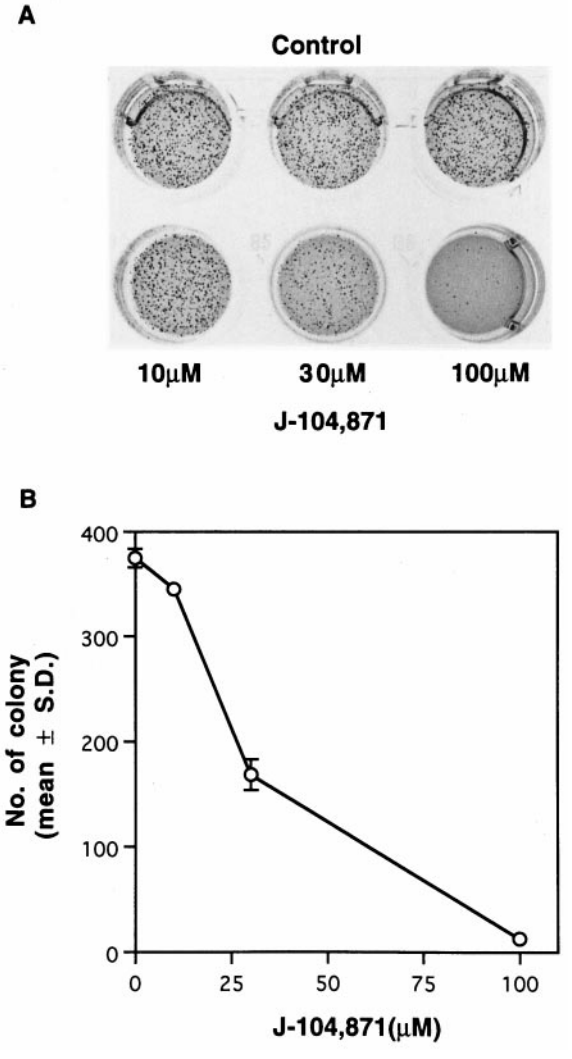

Fig. 5. J-104,871 suppresses colony formation. 5×10^3 cells of H-*ras*-transformed NIH3T3 were seeded on 24-well tissue culture dishes in 0.4 ml of 0.28% Noble agar (Difco) in DMEM containing 10% calf serum over 0.5 ml of 0.56% Noble agar in the same culture medium. Both layers contained 0.1% dimethyl sulfoxide or the indicated concentration of J-104,871. After 14 days, 0.2 ml of 0.5 mg/ml MTT in water was added and the agar was incubated overnight. A, Stained colonies were photographed. B, The number of stained colonies was analyzed with a colony counter. .

Discussion

In this report, the compound termed J-104,871 has been shown to be a novel and potent FTase inhibitor that competes with the isoprenoid substrate FPP. We modified our SS inhibitors to develop FTase inhibitors, because these two enzymes recruit the same substrate, FPP. Structural modifications have been implicated for advanced selectivity/potency for FTase rather than for SS (Iwasawa *et al.*, 1995, 1996; Aoyama *et al.*, 1998). J-104,871 was quite selective for FTase: both SS, the other major FPP-utilizing enzyme, and GGTase-I, the other CAAX prenyltransferase, were scarcely inhibited by this compound. The concentration of J-104,871 necessary to inhibit cellular processing by 50% is about 1000-fold higher than the concentration needed to inhibit farnesylation *in vitro*. Nevertheless, J-104,871 is one of the most potent cellular-active, FPP-competitive FTase inhibitors. Because some of the known FPP-competitive inhibitors with negatively charged structures have poor cell-level activity, due presumably to low cell penetrability (Gibbs *et al.*, 1993), and because our J-compound is suspected to have a similar nature owing to its tricarboxylic structure, it is surprising that J-104,871 exerted antitumor activity both *in vitro* and *in vivo*. It must be noted that suppression of cholesterol synthesis through SS inhibition would lead to the accumulation of

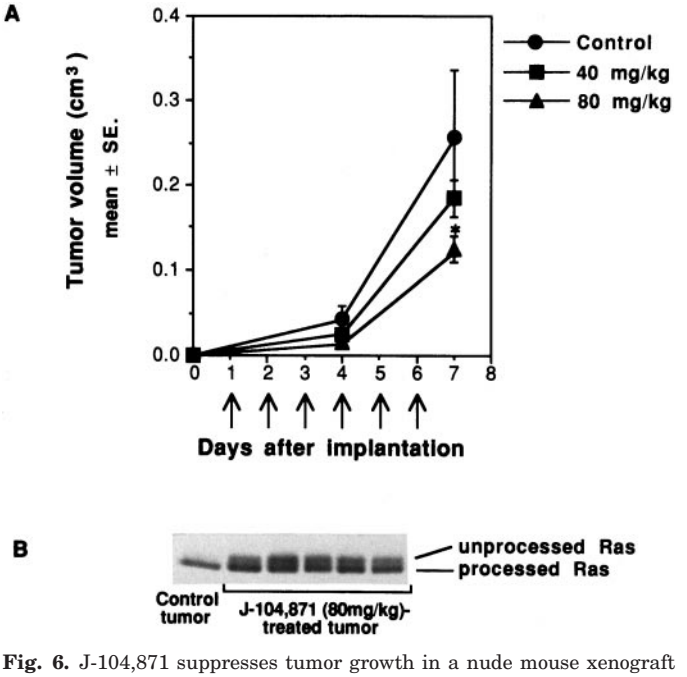

Fig. 6. J-104,871 suppresses tumor growth in a nude mouse xenograft model. On day 0, activated H-*ras*-transformed NIH3T3 cells were injected subcutaneously into the right flank of female nude mice, and the indicated doses of J-104,871 were administered intraperitoneally once daily for the following 6 days (five mice/group). A, Volume of the individual tumors was determined on days 4 and 7 as described in Experimental Procedures. Data are presented as the mean volume of five tumors. Statistical significance of the differences between the control and treated groups was evaluated using Student's *t* test (*, $p < 0.05$). *Arrows*, daily administration of J-104,871. B, On day 7, 24 hr after the last dose of 80 mg/kg of J-104,871, tumor tissues were excised from individual mice, lysed, and immunoblotted with anti-H-ras antibody as described in Experimental Procedures.

FPP, thereby abrogating the antitumor effects of FPP-competitive FTase inhibitors (Fig. 3C). J-104,871 had negligible potency against SS (Table 1), and thus did not inhibit cholesterol synthesis even at 100 μM (data not shown). This is a remarkable aspect of the present J-compound, and it distinguishes J-104,871 from previously reported FPP-competitive compounds that also inhibited SS activity (Gibbs *et al.*, 1993).

Conversely, as shown in Fig. 3, A and B, the FPP-competitive nature of the J-compound's inhibitory activity suggests that concomitant administration with HMG-CoA reductase inhibitors such as lovastatin may promote the antitumor efficacy of the J-compound.

It has been reported that several peptide-based inhibitors were effective against various human tumor cell lines, but they had a wide range of sensitivity in these cell lines (Nagasu *et al.*, 1995; Sepp-Lorenzino *et al.*, 1995). The level and type of Ras isoforms expressed in cells may contribute to differences in the sensitivity of these Ras-competitive inhibitors (James *et al.*, 1995; Rowell *et al.*, 1997; Zhang *et al.*, 1997). The inhibitory action of J-104,871 was influenced by the level of FPP but not that of Ras-peptide (Table 2, Fig. 3). Therefore, FPP-competitive inhibitors and Ras-competitive inhibitors may have different sensitivities and co-administration of both types of inhibitors may improve the antitumor spectrum of these compounds. We are now studying the efficacy of our J-series compounds against various human tumor cell lines. The effect of J-compounds in cells with K-*ras* mutations may also be of interest.

Acknowledgments

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