The Farnesyltransferase Inhibitor, FTI-2153, Blocks Bipolar Spindle Formation and Chromosome Alignment and Causes Prometaphase Accumulation during Mitosis of Human Lung Cancer Cells*

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Even though farnesyltransferase inhibitors (FTIs), a novel class of therapeutic agents presently in clinical trials, have preclinically outstanding anticancer activity and impressive lack of toxicity, their mechanism of action is not well understood. To enhance our understanding of how FTIs inhibit the growth of tumors, we have investigated their effects on cell cycle progression of two human lung cancer cell lines, A-549 and Calu-1. In this report, we show in synchronized A-549 and Calu-1 cells that FTI-2153 treatment resulted in a large accumulation of cells in the mitosis phase of the cell division cycle, with some cells in the G2/M phase. Furthermore, microtubule immunostaining and 4,6-diamidino-2-phenylindole DNA staining demonstrated that the FTI-2153-induced accumulation in mitosis is due to the inability of these cells to progress from prophase to metaphase. FTI-2153 inhibited the ability of A-549 and Calu-1 cells to form bipolar spindles and caused formation of monastral spindles. Furthermore, FTI-2153 induced a ring-shaped chromosome morphology and inhibited chromosome alignment. Time-lapse videomicroscopy confirmed this result by showing that FTI-2153-treated cells are unable to align their chromosomes at the metaphase plate. FTI-2153 did not affect the localization to the kinetochores of two farnesylated centromeric proteins, CENP-E and CENP-F. Thus, a mechanism by which FTIs inhibit progression through mitosis and tumor growth is by blocking bipolar spindle formation and chromosome alignment.

Protein farnesyltransferase catalyzes the covalent transfer of the lipid farnesyl from farnesyl pyrophosphate (FPP) to the cysteine thiol of proteins that end with a CAAX motif at their carboxy-terminal (C = cysteine, A = aliphatic amino acid, and X = any amino acid but preferably methionine and serine but not leucine) (1, 2). Protein farnesylation is required for the localization and function of several proteins pivotal to signal transduction pathways (1, 2). Among these is a family of low molecular weight GTPases called Ras (K-, N-, and H-Ras). Ras proteins transduce biological signals from cell surface receptors to the nucleus regulating important processes, including the cell division cycle, programmed cell death, and differentiation (3, 4). Furthermore, Ras proteins are found constitutively activated (due to point mutations) in about 30% of all human cancers, resulting in uncontrolled proliferation and tumor cell survival (5). The interest in farnesyltransferase was heightened when it was discovered that Ras requires farnesylation for its cancer-causing activity. This prompted many researchers to design farnesyltransferase inhibitors (FTIs) as potent anticancer drugs (6–8).

Intense research efforts over the last decade using several approaches have resulted in potent and selective FTIs (6–8). The approaches included rational design of CAAX peptidomimetics, FPP analogs, and FPP/CAAX bisubstrate transition state analogs as well as screening of natural product and chemical libraries. Some of the FTIs were shown to inhibit potently farnesyltransferase in vitro (IC50 in the picomolar range) and in whole cells (nanomolar range), to antagonize oncogenic H-Ras signaling, and to induce apoptosis in Ras-transformed fibroblasts when deprived of either serum or substratum attachment. More recently, FTIs were shown to induce apoptosis of attached human cancer cells in the presence of serum by a mechanism involving, at least in part, the phosphatidylinositol 3-kinase/Akt-2 survival pathway (9). Finally, FTIs have been shown to be potent inhibitors of tumor growth in several animal models. Their impressive antitumor activity and lack of toxicity to normal cells have led to ongoing human clinical trials with several FTIs (10).

Despite these major advances, the mechanism by which FTIs manifest their outstanding antitumor activity remains largely unknown. Although initially FTIs were hypothesized to inhibit tumor growth by inhibiting Ras farnesylation, several key observations suggested that farnesylated proteins other than Ras may be involved. First, the Ras mutation status does not predict sensitivity of human tumors to FTIs in soft agar assays (11). Second, K-Ras, the most prevalent mutated form of Ras in human cancers, becomes geranylgeranylated in the presence of attached human cancer cells in the presence of serum by a mechanism involving, at least in part, the phosphatidylinositol 3-kinase/Akt-2 survival pathway (9). Finally, FTIs have been shown to be potent inhibitors of tumor growth in several animal models. Their impressive antitumor activity and lack of toxicity to normal cells have led to ongoing human clinical trials with several FTIs (10).

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‡ The abbreviations used are: FPP, farnesyl pyrophosphate; FTI, farnesyltransferase inhibitor; PBS, phosphate-buffered saline; DAPI, 4,6-diamidino-2-phenylindole; MVA, mevalonic acid; H & E, hematoxylin & eosin.
One candidate, RhoB, another low molecular weight GTPase that is both farnesylated and geranylgeranylated, has been suggested as a target for FTI antitumor activity in fibroblasts (16). However, recent data in human cancer cells of epithelial origin argue against RhoB as a target, because both farnesylated and geranylgeranylated RhoB were shown to antagonize transformation and potently suppress human tumor growth in nude mice (17). Thus, to date, the critical target for FTIs has not been identified.

To further investigate the mechanism of action of FTIs we have studied the effects of FTIs on cell cycle progression of human cancer cells. Our goal is to identify the precise step of the cell cycle where a farnesylated protein or proteins is(are) required. Ultimately, we hope to identify such a protein using this strategy. Previously, we had shown that in NIH 3T3 murine fibroblasts protein farnesylation is not required for G1 to S phase transition (18). In contrast, in human cancer cells we had shown that FTIs can induce a G1 block, accumulate cells in G2/M or have no effect on cell cycle distribution, depending on the cell line (19). Here we show by flow cytometry, immunostaining, and time-lapse videomicroscopy, using synchronized human lung cancer cells, that FTIs induce a G1 and G2/M block and that the latter occurs through inhibition of bipolar spindle formation and chromosome alignment and causes a significant accumulation of the cells in prometaphase during mitosis.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—A-549 cells (ATCC, CCL-185) were maintained in Kaighn's F-12 medium, and Calu-1 cells (ATCC, HTB-54) were maintained in McCoy's medium. All media were adjusted to contain 1.5 g/liter sodium bicarbonate and were supplemented with 10% fetal bovine serum. All cells were maintained in a humidified incubator at 37 °C and 10% CO2.

**Flow Cytometry**—Cells were plated to subconfluency in 100-mm2 plates to obtain 5 x 10^5 to 1 x 10^6 cells for DNA analysis. After 24 h, the cells were treated with 30 μM lovastatin for 48 h and released with 2 mM mevalonic acid in the presence of vehicle (Me2SO) or 15 μM FTI-2153. Cells were harvested at indicated time points (0 h indicates time of release from lovastatin block) with trypsin (0.05%)/EDTA (0.5 mM), washed two times with PBS, resuspended in 500 μl of PBS, and fixed in 4.5 ml of 70% ethanol. Cells were stored in ethanol at −20 °C. When ready to stain with propidium iodide, cells were centrifuged to remove the ethanol and washed once in PBS. The cell pellet was then resuspended in 1 ml of PI/Triton X-100 staining solution (0.1% (w/v) Triton X-100 in PBS, 0.2 mg/ml RNase A, and 20 μg/ml propidium iodide) and
incubated at room temperature for at least 30 min. DNA analysis was done using a FACScan flow cytometer (Becton Dickinson, San Jose, CA) and ModFit LT 2.0 (Verity Software House, Topsham, ME).

Immunocytochemistry—Cells are grown on 2-well Lab-Tek II chamber slides (Nunc, Inc., Naperville, IL) to subconfluency and treated as described above. The cells are fixed in 50% methanol/4% paraformaldehyde (diluted with PBS from 16% stock (Electron Microscopy Sciences, Fort Washington, PA)) in a humidified chamber for 20 min at 4 °C and permeabilized with 0.5% Triton X-100 in PBS for 1 h. For microtubule immunocytochemistry, the fixed cells were stained with α-tubulin (clone B-5-1-2, Sigma T5168) diluted in 0.1% Tween 20, 1% bovine serum albumin in PBS for 1 h and fluorescein isothiocyanate-conjugated goat anti-mouse (Sigma F9006) diluted in 0.1% Tween 20, 1% bovine serum albumin in PBS for 25 min in the dark. For CENP-E and CENP-F immunostaining, a similar procedure was used except anti-CENP-E or anti-CENP-F antibodies (20) were used as primary antibodies and Alexa Fluor 594 goat anti-rabbit antibody (Molecular Probes, A-11012) was used for the secondary antibody. After final washes, slides were mounted with Vectashield mounting medium with DAPI (Vector Laboratories, Inc., Burlingame, CA) and viewed at magnifications of ×400 and ×1000 with an Orthoplan 2 fluorescence microscope (Leitz) and SmartCapture VP software (Digital Scientific).

Hematoxylin and Eosin Staining—The cells were fixed in B5 fixative for 2 h at room temperature. Cells were rinsed with water and incubated with Lugol’s iodine. After several rinses with reagent alcohol, cells were then stained with hematoxylin and eosin.

Time-lapse Videomicroscopy—Cells were plated at a density of 100,000 per 60-mm² plate and treated with lovastatin as for flow cytometry. Upon release of the cells from the lovastatin block, they were viewed using an Olympus K70 phase contrast microscope for up to 96 h.

Results

FTI-2153 accumulates synchronized human lung cancer cells in G₀/G₁ and G₂/M. To determine the effects of inhibition of protein farnesylation on cell cycle progression, we have used a standard method to synchronize cells (18). This was accomplished by blocking cells in G₁ by treatment with the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor lovastatin for 48 h and releasing the block by supplying mevalonic acid (MVA), the product of the reaction catalyzed by 3-hydroxy-3-methylglutaryl-coenzyme A reductase. The human cancer cells were either released in the presence or absence of the farnesyltransferase inhibitor FTI-2153 (21), as described under “Experimental Procedures.” Fig. 1A shows that, in the absence of FTI-2153, G₁-blocked A-549 cells exited G₁ 16–20 h after MVA release, entered and exited S phase between 20 and 32 h, entered and exited G₂/M between 24 and 36 h, re-entered a second cycle reaching G₁, S, and G₂/M peaks at 36, 40, and 44 h. In contrast, A-549 cells released in the presence of FTI-2153 were delayed in their release from the G₁ block by about 6 h, entered and exited the first S phase, and then entered G₂/M (Fig. 1A). Some cells accumulated in G₂/M but some exited and entered G₁. Although control cells had 59%, 26%, and 11% in
G1, S, and G2/M phases, respectively (96 h after MVA release), FTI-2153-treated cells had 42%, 9%, and 45% (Fig. 1C). A small proportion (2.5% and 1%) of cells contained 6N and 8N DNA peaks, but FTI-2153 treatment did not affect these proportions (Fig. 1C). Thus, FTI-2153 induced a delayed exit from G1 and accumulated A-549 cells in both a G1 and G2/M phases of the cell division cycle. Similar results were obtained with another human lung adenocarcinoma, Calu-1 (Fig. 1B). Here again, FTI-2153 induced a delay in the first cell cycle and resulted in accumulation of cells in G1 and G2/M. Calu-1 cells were able to exit G1, enter and exit S, and enter G2/M. However, a large proportion accumulated in G2/M (Fig. 1B). In contrast to A-549 cells, FTI-2153-treated Calu-1 cells had cells not only with 2N (G0/G1), 3N (S), and 4N (G2/M) DNA content but also accumulated cells with 6N and 8N DNA content (96 h after MVA release) (Fig. 1C). However, control cells had 54%, 28%, 12%, 4%, and 2% in G1, S, G2/M, 6N, and 8N peaks, respectively, FTI-2153-treated cells had 11%, 10%, 31%, 20%, and 28% 96 h after MVA release (Fig. 1C).

FTI-2153 Induces Accumulation of A-549 and Calu-1 Cells in Prometaphase during Mitosis—The demonstration that FTI-2153 resulted in accumulation of cells in G2/M suggested that protein farnesylation is critical for either entering G2, exiting G2, entering mitosis, or exiting mitosis. To determine the precise step at which inhibition of protein farnesylation results in disruption of the G2/M transition, we used immunostaining.

FIG. 3. FTI-2153 treatment induces ring-shaped morphology of chromosomes and inhibits bipolar spindle formation. A, H & E staining. Calu-1 cells were treated for 48 h and processed as described for Fig. 1B except that they were fixed and stained for H & E as described under “Experimental Procedures.” a, b, c, and d represent control Calu-1 cells undergoing prophase, metaphase, anaphase, and telophase/cytokinesis, respectively. e represents an FTI-2153-treated Calu-1 cell with its chromosomes in a ring-shaped morphology. B, DAPI staining. Calu-1 and A-549 cells were treated as in A except that they were DAPI-stained. The number of mitotic figures in prophase/prometaphase and those with their chromosomes arranged in a ring morphology were counted. The data are reported as average ± S.D. of percentages from three independent experiments. The differences between control and FTI-2153-treated cells were statistically different with p values for all samples less than 0.007. C, tubulin staining. Calu-1 and A-549 cells were treated as in A except that they were immunostained with an anti-α-tubulin antibody as described under “Experimental Procedures.” Mitotic figures from both control and FTI-2153 cells were counted and reported as average ± S.D. of percentages from three independent experiments. The differences between control and FTI-2153-treated cells were statistically different with p values for all samples less than 0.001.

FIG. 4. FTI-2153 treatment inhibits chromosome alignment of Calu-1 cells as visualized by time-lapse video microscopy. Progression through mitosis of Calu-1 cells treated as for flow cytometry was monitored using time-lapse videomicroscopy as described under “Experimental Procedures.” A, control; B, FTI-2153. The cell of interest is marked with a white asterisk. The DNA is indicated by a black arrowhead. Data are representative of two independent experiments.
techniques under the synchronization conditions of Fig. 1. Synchronized A-549 and Calu-1 cells were grown in the presence or absence of FTI-2153 and stained for tubulin and DAPI at different phases of the cell cycle. FTI-2153 treatment had no detectable effect on the distribution of chromatin and microtubules at interphase when compared with untreated cells (Fig. 2). In the absence of FTI-2153 treatment, Fig. 2 (A and B) shows that Calu-1 and A-549 cells traverse all phases of mitosis. In prophase the nuclear membrane is disassembled, DNA is condensed, the spindle poles have begun to separate, and mitotic bipolar spindle assembly has begun. At this point, asters radiating from bipolar centrosomes have formed. In metaphase, the chromosomes are aligned at the metaphase plate, and the mitotic bipolar spindles are fully formed. At anaphase the spindles begin to depolymerize, pulling the chromosomes toward each pole. By telophase the chromosomes have reached their respective poles, and the cell begins to undergo cytokinesis. In contrast, FTI-2153-treated cells accumulated in mitosis with chromosomes organized into a ring-like shape (Fig. 3 A). We counted the ring shape mitotic figures in control and treated cells. In Calu-1 cells, out of 53 control prometaphase cells, 30% and out of 56 FTI-2153-treated prometaphase cells, 86% had their chromosomes in a ring-like shape, respectively. Similarly, in A-549 cells (39 control and 59 FTI-2153-treated prometaphase cells) 5% and 68% of control and FTI-2153-treated cells in prometaphase during mitosis.

**FTI-2153 Inhibits Chromosome Alignment**—The data described above suggest that a farnesylated protein is critical to a step between prophase and metaphase involving chromosome and/or spindle organization. We first determined the effects of FTI-2153 on organization of chromosomes by three methods, H&E staining, DAPI staining, and time-lapse videomicroscopy.

**H & E Staining**—For H&E staining, cells were treated as described under experiments of Figs. 1 and 2, were harvested at 48 h, fixed with B5 fixative, and stained with H&E to visualize DNA. Fig. 3A shows that in vehicle-treated Calu-1 cells, chromatin condenses at prophase, aligns at metaphase, begins to separate at anaphase, and reaches the cellular poles at telophase/cytokinesis. In contrast, in FTI-2153 cells, these chromatin figures are rarely seen. Instead, chromosomes in these cells are organized into a ring-like shape (Fig. 3A). We counted the ring shape mitotic figures in control and treated cells. In Calu-1 cells, out of 53 control prometaphase cells, 30% and out of 56 FTI-2153-treated prometaphase cells, 86% had their chromosomes in a ring-like shape, respectively. Similarly, in A-549 cells (39 control and 59 FTI-2153-treated prometaphase cells) 5% and 68% of control and FTI-2153-treated cells in prometaphase during mitosis.

**FTI-2153 Blocks Bipolar Spindle Formation and Chromosome Alignment**—Data are representative of two independent experiments.
prometaphase cells had this shape, respectively.

**DAPI Staining**—To further characterize these effects of FTI-2153 on chromatin structure, we stained the DNA with DAPI as described under “Experimental Procedures.” For each sample at least 38 and as many as 139 mitotic figures at prometaphase were counted. Fig. 3B shows that, in control A-549 cells, 79.0% and 21.0% had normal and ring shapes, respectively, whereas FTI-2153-treated A-549 cells had 26.0% and 74.0% normal and ring-shaped chromosomes, respectively. Similar results were obtained with Calu-1 cells where FTI-2153 increased the ring-shaped chromosome morphology from 27.9% to 71.6% (Fig. 3B).

Time-lapse Videomicroscopy—The above studies clearly demonstrate that, in both Calu-1 and A-549 cells, FTI-2153 interferes with a step leading to prometaphase involving chromosome alignment. However, a drawback of these studies is that they only reflect pictures of what happened every 4 h. Yet the whole process of mitosis occurs around 1–2 h for most cells. To follow in a continuous fashion the effects of FTI-2153 on the progression through mitosis, we performed time-lapse videomicroscopy. To this end, A-549 and Calu-1 cells, which had been synchronized and released in the presence or absence of FTI-2153, were recorded. Several cells were observed for up to 70 h after MVA release. Figs. 4 and 5 show the cell cycle progression for one cell per sample. In the absence of FTI-2153, Calu-1 cells progressed normally through mitosis (Fig. 4A). For the cell shown (marked with the white star) mitosis started about 35.5 h after MVA release. At this time, the cell rounded and its DNA (black arrow) condensed initiating prophase. The chromosome then lined up along the metaphase plate, separated during anaphase, and migrated toward the two opposing poles (see two black arrows). The cells then pinched off in the middle during telophase/cytokinesis. After completion of mitosis, the resulting two daughter cells (two white stars) flattened out again. The average length of mitosis for the 12 cells (from two independent experiments) analyzed was 56.8 ± 12.6 min. In contrast, FTI-2153-treated cells behaved quite differently. The cell followed (white star) was able to round up 45 h after MVA release (Fig. 4B). However, progression through mitosis did not occur, but the cells eventually flattened back out. No apparent metaphase, anaphase, or telophase was observed. The chromosomes did not align on the metaphase plate. In the 10 cells (two independent experiments) observed, the cells were able to flatten out again after an average of 286.3 ± 110.4 min. Therefore, FTI-2153-treated Calu-1 cells appear to synthesize DNA, but they are not able to actually divide. These results are consistent with those of Fig. 1B where FTI-2153 accumulated Calu-1 cells in mitosis. Furthermore, because these cells are not able to divide, cells with more than 4N DNA should be more prevalent in FTI-2153-treated cells. We have confirmed this by flow cytometry where 20% and 28% of Calu-1 cells had 6N and 8N DNA, respectively, in FTI-2153-treated samples but only 4% and 2% in control sample (Fig. 1C).

As was the case for Calu-1 cells, A-549 cells that were not treated with FTI-2153 were able to traverse all phases of mitosis. Fig. 5A shows that one of these A-549 cells (white star) rounded up to initiate prophase at 68 h after MVA release, aligned its chromosomes (black arrow) at metaphase, and separated them at anaphase. The cell then pinched off into two daughter cells during telophase/cytokinesis (Fig. 5A). The two daughter cells then flattened out. The average time it took 18 cells (from two independent experiments) to go through mitosis was 58.7 ± 15.1 min. A-549 cells released in the presence of FTI-2153 did round up but never went through metaphase, anaphase, or telophase (Fig. 5B). However, out of the 12 cells (from two independent experiments) observed, 11 actually divided and the two daughter cells flattened (Fig. 5B). The average time for the 11 cells to divide is 83.0 ± 25.6 min.

**FTI-2153 Inhibits Bipolar Spindle Formation but Has No Effect on CENP-E and -F Localization**—The data described so far demonstrate that inhibition of protein farnesylation results in accumulation of cells in a prometaphase-like state and suggest disruption of interactions between the spindle and chromosomes or perturbation of spindle assembly. CENP-E, a kinetochore-associated microtubule motor that is important for chromosome alignment (20, 22), contains a potential farnesylation site at its carboxyl terminus. Because the kinetochore-targeting domain of CENP-E is also located at its carboxyl terminus (23), farnesylation might be important for kinetochore binding. We therefore examined the effect of FTI-2153 treatment on the distribution of CENP-E in Calu-1 and A-549 cells that were in mitosis. Immunofluorescence staining shows that CENP-E accumulates at the kinetochores of chromosomes in both control prometaphase cells and drug-treated cells that were arrested in a prometaphase-like state (Fig. 6). Thus, FTI-2153 does not affect the ability of CENP-E to bind to kinetochores. Similar results were obtained for CENP-F (data not shown), another kinetochore protein that has a potential farnesylation site at its carboxyl terminus (24).

We next determine whether FTI-2153 affected bipolar spindle formation. We treated A-549 and Calu-1 cells as described for the experiments of Fig. 2, immunostained with an antibody against α-tubulin and counted mono and diaster figures during prometaphase for both vehicle- and FTI-2153-treated cells. For every sample, at least 33 and as many as 75 mitotic figures were counted. Fig. 3C shows that, in the absence of FTI-2153, 67.1 ± 0.7% (average of three experiments) of the Calu-1 mitotic figures counted had diasters. In contrast, in FTI-2153-treated Calu-1 cells only 2.3 ± 2.7% of the mitotic figures counted had diasters. Similarly, Fig. 3C shows that, in A-549 cells treated with vehicle, 76.4% and 87.0% (two experiments) whereas, in those treated with FTI-2153, only 18.2% and 18.9% of the mitotic figures at prometaphase had diasters. FTI-2153 treatment increased the percentage of mitotic figures with monoaasters from 32.9 ± 0.7% to 97.7 ± 2.7% in Calu-1 cells (Fig. 3C). Similarly, FTI-2153 increased monoaaster formation from 13% and 23.6% to 81.8% and 81.1% in A-549 cells. Thus, FTI-2153 inhibited bipolar spindle formation in both A-549 and Calu-1 cells.

**DISCUSSION**

Our previous results demonstrated that FTIs induce a G2/M accumulation in some human cancer cells, but the precise stage of the cell cycle where cells are arrested was not known (19). In this manuscript we present data demonstrating that inhibition of protein farnesylation blocks cells in prometaphase. FTI-2153-treated Calu-1 and A-549 cells were able to enter mitosis as evident from cell rounding, chromosome condensation, and nuclear membrane disassembly. However, these cells were not able to form bipolar spindles and their chromosomes failed to form a metaphase plate. Thus, although FTIs disrupt mitosis, they are distinct from other anti-cancer drugs, such as Taxol, that stabilize tubulin polymerization and block mitosis at the latter stage of metaphase to anaphase transition (25). FTI treatment outcome appears to be more similar to tubulin polymerization-disrupting agents, such as vinblastine, which disturb events leading to metaphase (26).

FTI-2153-treated Calu-1 cells accumulated in prometaphase and were not able to divide (Fig. 4). However, these cells were not permanently arrested in mitosis, because they eventually exit mitosis without dividing to produce 6N- and 8N-containing cells. This was confirmed by the flow cytometry data of Fig. 1C where, after prolonged FTI-2153 treatment (96 h), 20% and...
28% of the Calu-1 cells contained 6N and 8N DNA content, respectively, as compared with 4% and 2% with untreated cells. In A-549, where cells were able to divide even in the presence of FTI-2153, the percentage of cells containing 6N and 8N DNA is only 2.6% and 0.9%, respectively. Thus, even though in human lung adenocarcinoma cells protein farnesylation appears to be pivotal for the proper progression through mitosis, A-549 but not Calu-1 cells are able to adapt and divide despite the fact that they are not able to form bipolar spindles. It is possible, however, that at higher concentrations of FTI-2153, A-549 would not be able to divide.

Our results suggest that a farnesylated protein(s) is (are) involved in a step critical to bipolar spindle formation and chromosome alignment. We examined the possibility that FTI-2153 arrested cells in mitosis by interfering with the localization of the kinetochore proteins CENP-E and CENP-F, because both proteins contained potential farnesylation sites. However, FTI-2153 treatment did not affect the ability of these proteins to bind to kinetochores during mitosis. This is consistent with a recent study (reported while this work was being reviewed) that shows that another FTI, SCH66336, did not affect the localization of CENP proteins (27). Although SCH66336 did not affect CENP-E localization, the authors suggested that CENP-E might be involved based on the ability of SCH66336 to inhibit the association of CENP-E with microtubules (27). It is possible that FTIs could interfere with the functions of these proteins when they are bound to kinetochores. In the case of CENP-E, its carboxyl terminus has been shown to bind microtubules in vitro (22), and, thus, inhibition of farnesylation might affect its ability to interact with microtubules in vitro. Despite this possibility, we do not believe that the mitotic arrest induced by FTI-2153 is due to disruption of CENP-E function at kinetochores. It has been shown that the primary defect in cells that are deficient for CENP-E function is the inability to align chromosomes properly at the metaphase plate. These cells arrest in mitosis for extended periods of time with their chromosomes distributed in a characteristic pattern with some positioned near one of the two separated poles while others lie in the center of the spindle (20, 23, 28, 29). This pattern is distinctly different from the rosette configuration that is seen in FTI-treated cells. Furthermore, both in vitro and in vivo experiments show that CENP-E is not essential for spindle pole separation, because cells that lack CENP-E function are still able to establish a bipolar spindle (20, 23, 28–30). This outcome is clearly also distinct from that seen for FTI-treated cells where spindle poles failed to separate. Therefore, if CENP-E is the target, the FTI-treated cells should have arrested with a bipolar spindle but unaligned chromosomes.

Based on the large number of cells that accumulated in mitosis with unseparated spindle poles, we believe that the primary targets of FTI-2153 are components that are essential for separation of the spindle poles. It is noteworthy that disruptions of cytoplasmic dynein as well as the Eg5 kinesin-related protein all arrested cells in mitosis with unseparated spindle poles that were surrounded by a ring of chromosomes (31, 32). Indeed, Eg5 has recently been shown to be the target for Monastrol, a compound that was isolated based on its ability to arrest cells in mitosis and that was subsequently shown to inhibit bipolar spindle formation (33). However, neither cytoplasmic dynein nor Eg5 contain the consensus CAAX motif that specifies isoprenylation. Thus, proteins that interact with dynein or Eg5 or those involved in their regulation in cancer cells should be prominent candidates for FTIs. A potential candidate is H-Ras, which is exclusively farnesylated and is known to activate the cyclin-dependent kinase, cdc2, which in turn phosphorylates Eg5 to promote bipolar spindle formation.

Thus, the results presented in this report enhance our understanding of how inhibition of protein farnesylation results in the accumulation of cells in the mitotic phase of the cell division cycle. This appears to involve a critical step for bipolar spindle formation and chromosome alignment resulting in accumulation of cells in prometaphase during mitosis. The results implicate protein farnesylation in the regulation of pathways pivotal to the prophase/metaphase transition.

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