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The farnesyltransferase inhibitor, FTI-2153, inhibits bipolar spindle formation during mitosis independently of transformation and Ras and p53 mutation status

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Abstract

Recently, we have shown that the farnesyltransferase inhibitor FTI-2153 induces accumulation of two human lung cancer cell lines in mitosis by inhibiting bipolar spindle formation during prometaphase. Here we investigate whether this mitotic arrest depends on transformation, Ras and/or p53 mutation status. Using DAPI staining (DNA) and immunocytochemistry (microtubules), we demonstrate that in normal primary foreskin fibroblasts (HFF), as well as in several cancer cell lines of different origins including human ovarian (OVCAR3), lung (A-549 and Calu-1) and fibrosarcoma (HT1080), FTI-2153 inhibits bipolar spindle formation and induces a rosette morphology with a monopolar spindle surrounded by chromosomes. In both malignant cancer cell lines and normal primary fibroblasts, the percentage of prometaphase cells with bipolar spindles decreases from 67–92% in control cells to 2–28% in FTI-2153 treated cells. This inhibition of bipolar spindle formation correlates with an accumulation of cells in prometaphase. The ability of FTI-2153 to inhibit bipolar spindle formation is not dependent on p53 mutation status since both wild-type (HFF, HT1080 and A-549) and mutant (Calu-1 and OVCAR3) p53 cells were equally affected. Similarly, both wild-type (HFF and OVCAR3) and mutant (HT1080, Calu-1 and A-549) Ras cells accumulate monopolar spindles following treatment with FTI-2153. However, two cell lines, NIH3T3 (WT Ras and WT p53) and the human bladder cancer cell line, T-24 (mutant H-Ras and mutant p53) are highly resistant to FTI-2153 inhibition of bipolar spindle formation. Finally, the ability of FTI-2153 to inhibit tumor cell proliferation does not correlate with inhibition of bipolar spindle formation. Taken together these results demonstrate that the ability of FTI-2153 to inhibit bipolar spindle formation and accumulate cells in mitosis is not dependent on transformation, Ras or p53 mutation status. Furthermore, in some cell lines, FTIs inhibit growth by mechanisms other than interfering with the prophase/metaphase traverse.
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Keywords: FTase; FTIs; mitosis; bipolar spindles; Ras; p53

Abbreviations: CAAX, C=cysteine, A=aliphatic amino acid, X=any amino acid; FTase, farnesyltransferase; FTI, farnesyltransferase inhibitor; GGTase, geranylgeranyltransferase; HFF, human foreskin fibroblasts

Introduction

Protein farnesyltransferase (FTase) catalyzes the covalent attachment of the lipid farnesyl to the cysteine sulfhydryl of proteins that terminate at their carboxyl terminus with the consensus sequence CAAX (C=cysteine, A=aliphatic amino acid, X=methionine, serine, alanine, glutamic acid or cysteine) (reviewed in Zhang and Casey).[1] Because farnesylation was shown to be required for the malignant transforming activity of the oncoprotein Ras, FTase inhibitors (FTIs) were developed as potential anticancer drugs.[2–4] *In vitro*, FTIs are highly potent and selective for FTase over the closely related family member, protein geranylgeranyltransferase-I (GGTase I).[2] In cultured cells, FTIs inhibit the growth of transformed cells selectively over non-transformed cells.[5,6] Furthermore, FTIs are very effective at inhibiting the growth of a range of human cancer cell lines in soft agar as well as in nude mouse xenografts.[7–13] This tumor growth inhibition does not depend on the Ras mutation status and several lines of evidence suggest that other farnesylated proteins, in addition to Ras, may be critical targets for FTIs' mechanism of action.[9,13] FTIs are also highly effective at inducing tumor regression in transgenic animal models.[14–17] Because of their outstanding antitumor activity and lack of toxicity in animal models, FTIs have rapidly entered human clinical trials and some are in phase III.[18,19]

Although major milestones have been reached in the use of FTIs to treat cancer, the mechanism of their antitumor activity is still not known, in part because of about 50 known potential substrates for FTase. Therefore, depending on which farnesylated proteins are expressed within a cancer cell and contribute to its malignancy as well as the genetic background of the cell, FTIs may or may not be effective. Furthermore, in those cellular environments

where FTIs are effective, the mechanisms by which they inhibit tumor cell growth may be different. Consistent with this are our previous cell cycle studies in a panel of human cancer cell lines, that showed that FTIs either induce a G0/G1 phase accumulation, a G2/M phase accumulation or have no effect on cell cycle distribution.[20] Recently, we[21] and others[22] have focused on understanding the mechanism by which FTIs induce G2/M accumulation. In two human cancer cell lines where FTIs induce G2/M accumulation, we found that FTIs inhibit bipolar spindle formation and prevent chromosome alignment, thus accumulating cells at prometaphase during mitosis.[21] This suggested that a farnesylated protein plays a pivotal role in the regulation of the transition from prophase to metaphase. However, these studies were performed with two human lung cancer cell lines only and whether the requirement for farnesylated proteins for prophase/metaphase transition applies to other cancer cell lines with different genetic alterations is not known. Furthermore, normal cells are known to be relatively insensitive to FTIs. Therefore, it is also important to determine whether FTIs inhibit bipolar spindle formation in non-transformed cells. In this manuscript, we report the use of a panel of human cancer cell lines, human primary foreskin fibroblasts and the immortalized cell line NIH3T3 to address these important questions.

Results

Recently, we have shown that FTI-2153 inhibits bipolar spindle formation in two human lung cancer cell lines suggesting that farnesylated proteins are required for the prophase/metaphase transition. In this manuscript we have expanded these studies to other cell lines to investigate the importance of the Ras and p53 mutation status as well as whether non-transformed cells also require farnesylated proteins for bipolar spindle formation. To this end, we have used a panel of human cancer cell lines, human primary fibroblasts and NIH3T3 cells. Table 1 lists the cells used as the human cancer cell lines OVCAR3 (ovarian), T-24 (bladder), HT1080 (fibrosarcoma), the two human lung adenocarcinoma cell lines, A-549 and Calu-1 (that were used in the previous study), NIH3T3 cells (immortalized murine fibroblasts) and HFF (human foreskin primary fibroblasts).

Table 1

Cell line	Tissue type	Ras status	p53 status
NIH3T3	Immortalized mouse fibroblasts	WT	WT
HFF	Primary human foreskin fibroblasts	WT	WT
HT1080	Human fibrosarcoma	mutant N-Ras	WT
T-24	Human bladder carcinoma	mutant H-Ras	mutant
OVCAR3	Human ovarian carcinoma	WT	mutant
A-549	Human lung adenocarcinoma	mutant K-Ras	WT
Calu-1	Human lung adenocarcinoma	mutant K-Ras	deleted

WT, wild-type

Effects of FTI-2153 treatment on progression through mitosis

The different cell lines (Table 1) were treated for 48 h with FTI-2153 and processed with DAPI staining to visualize the DNA and anti-α-tubulin antibody to immunolocalize microtubules, as described under Materials and Methods. First we determined the effect of FTI-2153 on progression through various phases of mitosis. Figure 1 shows control, non-treated A-549 cells undergoing cell division by traversing the various mitotic phases which were defined as follows: prophase/prometaphase cells have condensed chromatin that was not yet aligned on the metaphase plate, metaphase cells have the chromosomes aligned on the central axis between the two poles, anaphase cells have their chromosome being pulled toward the poles and telophase/cytokinesis cells have the DNA at the poles and are pinched at the central axis (Figure 1). When A-549 cells were treated with FTI-2153 (15 μM for 48 h), the proportion of cells at prometaphase increased relative to the other phases of mitosis. Furthermore, FTI-2153

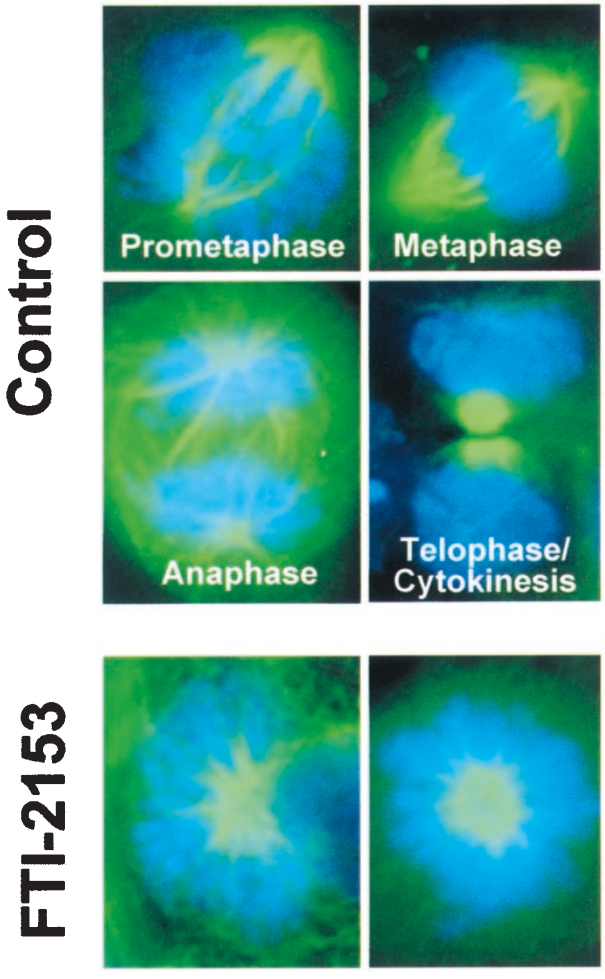


Figure 1 FTI-2153 induces accumulation of a rosette-like chromatin/microtubule morphology during mitosis. A-549 cells were treated with either vehicle (control) or FTI-2153 (15 μM, 48 h) and the cells were stained with DAPI to mark the DNA (blue) and with an antibody against α-tubulin to label microtubules (green) as described in Materials and Methods

accumulated cells at prometaphase with a rosette-like morphology where chromosomes form a ring surrounding a monoaster of microtubules (Figure 1). We next quantitated the effects of FTI-2153 on mitotic phase distribution in various cell lines. Figure 2 shows that in all cells, except for T-24 and NIH3T3, FTI-2153 treatment increased the proportion of mitotic cells in prometaphase and decreased the percentage of cells in telophase/cytokinesis. For example, in HT1080 cells, the percentage of cells in prometaphase and telophase/cytokinesis were 5 and 85% in control cells and 55 and 35% in FTI-2153-treated cells, respectively. Similarly, in HFF cells the percentages changed from 10 and 85% in control cells to 60 and 30% in FTI-2153-treated cells. Calu-1 and A-549 cells, as described previously, had similarly large changes (from 15.6 and 35% to 55.7 and 17% in Calu-1 cells and from 2.4 and 78.9% to 45.9 and 37.8% in A-549 cells), whereas OVCAR3 had smaller changes (from 33 and 42% to 56 and 26%). In contrast, FTI-2153 did not significantly affect the distribution of the different phases of mitosis in T-24 and NIH3T3 cells (Figure 2).

FTI-2153 inhibits bipolar spindle formation in normal and malignant cells regardless of their Ras and p53 mutation status

The FTI-2153-induced prometaphase accumulation suggested that FTIs affect a step in mitosis that is critical to

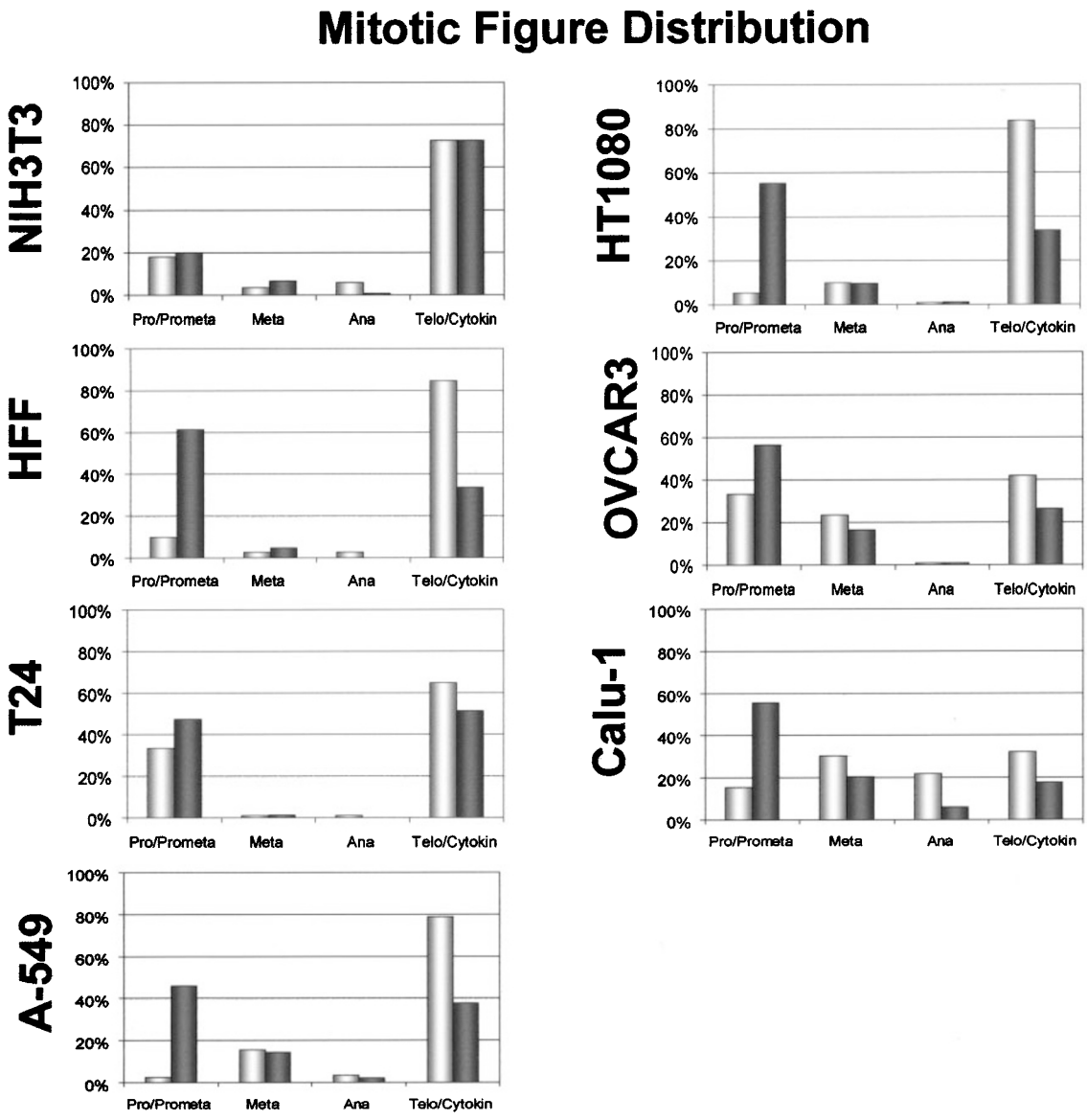


Figure 2 FTI-2153 accumulates cells in prometaphase independently of transformation, Ras and p53 mutation status. Asynchronous populations of cells were treated with 15 μM FTI-2153. After 72 h, cells were stained as described for Figure 1 and at least 50 mitotic figures for each cell type were counted and categorized as prophase/prometaphase, metaphase, anaphase or telophase/cytokinesis. Control cells are indicated as white bars and FTI-treated cells are indicated as gray bars

reaching metaphase. In order for cells to reach metaphase, centrosomes must separate to form bipolar spindles prior to chromosome alignment at the metaphase plate. We therefore determined whether FTI-2153 affects bipolar spindle formation and chromatin morphology. To this end, the various cells of Table 1 were treated with FTI-2153 as described for Figures 1 and 2 and the number of cells with monoasters *vs* those with diasters were counted. The percentages of the prometaphase cells with monoasters *vs* diasters were reported as mean $\pm$ S.D. of three independent experiments except for OVCAR3, A-549 and Calu-1 cells. In each experiment at least 50 prometaphase cells were counted. Figure 3 shows that in HT1080 cells, the percentage of prometaphase cells with a monoaster increased from 8.0 $\pm$ 7.9% in control cells to 71.4 $\pm$ 18.6% in FTI-2153-treated cells. In these cells, the percentage with diasters decreased from 92.0 $\pm$ 7.9 to 28.6 $\pm$ 18.6% upon FTI-2153 treatment. In HFF cells the percentage of prometaphase cells with diasters decreased from 75.2 $\pm$ 10.1 to 26.1 $\pm$ 20.4%. Figure 3 also shows that in OVCAR3 the percentage of cells with diasters decreased from 73.6 to 20.4%. In A-549 cells the decrease was from 81.5 to 34.0% and in Calu-1 cells the decrease was from 95 to 0%. In contrast, the percentage of prometaphase cells with diasters was not affected by FTI-2153 treatment in NIH3T3 (from 83.2 $\pm$ 3.4 to 72.9 $\pm$ 10.3%) and T-24 cells (from 76.7 $\pm$ 18.5 to 75.7 $\pm$ 8.6%). These results clearly demonstrate that the ability of FTI-2153 to inhibit bipolar spindle formation is independent of whether the cell is transformed, of tissue of origin, and of Ras and p53 mutation status.

FTI-2153 increases the percentage of prometaphase cells with ring-like DNA morphology in transformed and non-transformed cells

The ability of FTI-2153 to inhibit bipolar spindle formation is also accompanied by a rosette-like morphology where

Bipolar Spindle Formation

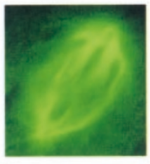
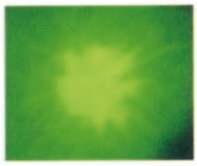
Cell Line	Sample	Average	
			
NIH3T3	Control	83.2 ± 3.4%	16.8 ± 3.4%
	FTI-2153	72.9 ± 10.3%	27.1 ± 10.3%
HFF	Control	75.2 ± 10.1%	24.8 ± 10.1%
	FTI-2153	26.1 ± 20.4%	73.8 ± 20.4%
HT1080	Control	92.0 ± 7.9%	8.0 ± 7.9%
	FTI-2153	28.6 ± 18.6%	71.4 ± 18.6%
T24	Control	76.7 ± 18.5%	23.3 ± 18.5%
	FTI-2153	75.7 ± 8.6%	24.2 ± 8.6%
OVCAR3	Control	73.6%	26.4%
	FTI-2153	20.4%	79.7%
A-549	Control	81.5%	18.5%
	FTI-2153	34.0%	66.0%
Calu-1	Control	95.00%	5.00%
	FTI-2153	0.00%	100.00%

Figure 3 FTI-2153 inhibits bipolar spindle formation independently of transformation, Ras and p53 mutation status. Asynchronous populations of cells were treated with 15 μM FTI-2153. After 72 h, cells were stained with an antibody against α-tubulin to mark the microtubules. At least 50 prometaphase cells were counted and analyzed for bipolar spindle formation

the monoaster is surrounded by a ring of chromosomes. To determine if accumulation of prometaphase cells with ring chromosome morphology is also independent of transformation as well as Ras and p53 mutation status, the cells in Table 1 were processed as described above for Figures 1 and 2. Figure 4 shows that in HT1080 cells the percentage of cells with ring-like DNA morphology increased from 15.4±12.2 to 66.3±9.7% upon FTI-2153 treatment. This percentage increased from 22.5±11.0 to 69.9±11.7% in HFF cells, from 19.3 to 48.9% in OVCAR3 cells, from 28.6 to 85% in Calu-1 cells and from 20.7 to 75.7% in A-549 cells. In contrast, in T-24 cells (20.2±19.9 to 24.7±9.2%) and NIH3T3 cells (22.0±9.3 to 29.7±12.6%) FTI-2153 had little effect on the percentage of cells with ring-like chromatin morphology.

The ability of FTI-2153 to inhibit tumor cell proliferation is independent of its ability to inhibit bipolar spindle formation

We next determined whether there is a correlation between FTI-2153 inhibition of tumor cell growth and inhibition of bipolar spindle formation. To this end, NIH3T3, HFF, HT1080, A-549, OVCAR3, T-24 and Calu-1 cells were treated in 96-well plates with various concentrations of FTI-2153 (0–50 μM) for 48 h. Inhibition of tumor cell proliferation was assessed by MTT assay as described under Materials and Methods. Figure 5 shows that some cell lines are relatively resistant to FTI-2153 (NIH3T3, HFF and HT-1080), others are more sensitive (T-24 and Calu-1) and some are in between (A-549 and OVCAR3). At 15 μM, FTI-2153 inhibited T-24 and Calu-1 cell growth by 38 and 36%, respectively. In contrast,

DNA Morphology

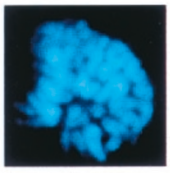
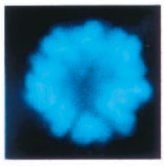
Cell Line	Sample	Average	
			
NIH3T3	Control	78.0% ± 9.3	22.0% ± 9.3
	FTI-2153	70.3% ± 12.6	29.7% ± 12.6
HFF	Control	77.5% ± 11.0	22.5% ± 11.0
	FTI-2153	30.1% ± 11.7	69.9% ± 11.7
HT1080	Control	84.6% ± 12.2	15.4% ± 12.2
	FTI-2153	33.7% ± 9.7	66.3% ± 9.7
T24	Control	79.8% ± 19.9	20.2% ± 19.9
	FTI-2153	75.3% ± 9.2	24.7% ± 9.2
OVCAR3	Control	80.8%	19.3%
	FTI-2153	51.1%	48.9%
A-549	Control	79.3%	20.7%
	FTI-2153	24.3%	75.7%
Calu-1	Control	71.4%	28.6%
	FTI-2153	15.0%	85.0%

Figure 4 FTI-2153 induces a ring-like chromatin structure independently of transformation, Ras and p53 mutation status. Asynchronous populations of cells were treated with 15 μM FTI-2153. After 72 h, cells were stained with DAPI to mark the DNA. At least 50 prometaphase cells were counted and analyzed for ring-like chromatin morphology

NIH3T3, HFF and HT-1080 were less sensitive and were inhibited by only 8, 8 and 13%, respectively. A-549 and OVCAR3 cell growth was inhibited by 25 and 22%, respectively. Thus, even though T-24 and Calu-1 cells are equisensitive to FTI-2153 cell growth inhibition, FTI-2153 inhibits bipolar spindle formation only in Calu-1 cells (Figure 3). Similarly, HFF and NIH3T3 cells are both resistant to FTI-2153 growth inhibition (Figure 5), yet only NIH3T3 cells are resistant to FTI-2153 inhibition of bipolar spindle formation (Figure 3).

Discussion

We have investigated whether the ability of FTI-2153 to inhibit bipolar spindle formation and induce mitotic arrest depends on transformation, and/or Ras and p53 mutation status. Because FTIs are relatively non-toxic to normal cells, a key issue is whether FTIs have similar effects on non-transformed immortalized cells and normal primary cells. Furthermore, a critical question concerns the inhibition of bipolar spindle formation and its dependency on key genetic aberrations associated with malignant transformation, such as Ras and p53 deletion/mutation status. Using a panel of human cancer cell lines of different tissue origins and with different genetic alterations, a normal primary foreskin fibroblast and the immortalized mouse fibroblast cell lines, NIH3T3, we have demonstrated that FTI-2153 inhibits bipolar spindle formation in non-transformed cells (i.e. HFF). This is a highly significant finding that indicates that the farnesylated proteins that mediate prometaphase transition during mitosis are also present in untransformed cells such as the primary foreskin fibroblasts HFF. Furthermore, the tissue of origin is not important since these effects were seen with HT1080 (fibrosarcoma), A-549 and Calu-1 (lung carcinoma), and OVCAR3 (ovarian carcinoma). More importantly, the effects are independent of the Ras mutation status since they were seen in cancer cell lines with both wild-type Ras (OVCAR3) and mutated Ras (A-549 (K-Ras), Calu-1 (K-Ras), and HT1080 (N-Ras)). Similarly, the ability of FTI-2153 to inhibit bipolar spindle formation is independent of the p53 deletion/mutation status since Calu-1 (deleted p53), OVCAR3 (mutated p53) and HFF, HT1080, A-549 (WT p53) are similarly affected. This is consistent with work published by Ashar *et al*.[22] However, FTI-2153 did not inhibit bipolar spindle formation in two cell lines: T-24, a bladder carcinoma with H-Ras and p53 mutations and NIH3T3 cells with WT Ras and p53. At this stage the mechanism by which these two cell lines escape the FTI effects is not known. Consistent with this data, FTI-2153 does not induce prometaphase accumulation in these cell lines.

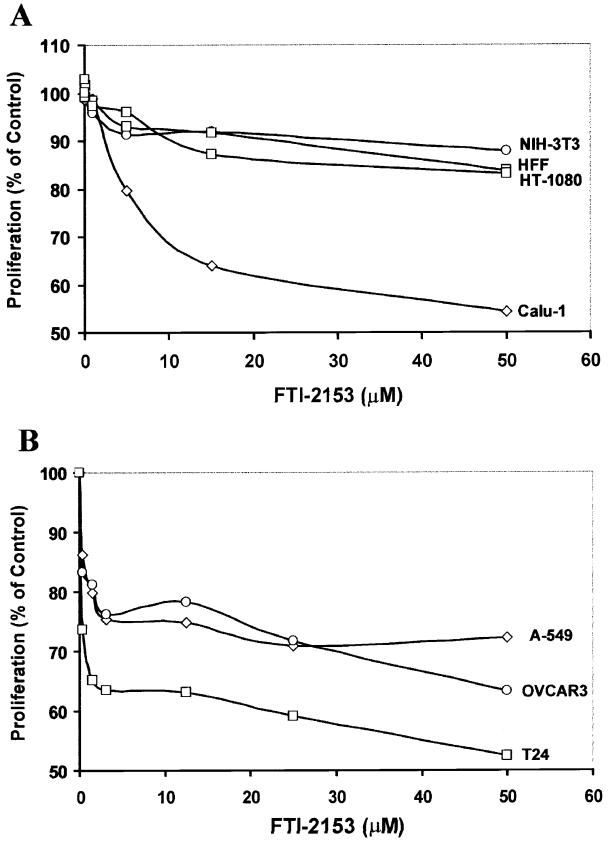


Figure 5 Tumor cell growth inhibition by FTI-2153. Cells were plated in 96-well plates and treated with various concentrations of FTI-2153. Inhibition of cell proliferation was determined by MTT assay as described under Materials and Methods

Another important finding of our studies is that inhibition of tumor cell proliferation does not require inhibition of bipolar spindle formation. For example the growth of both T-24 and Calu-1 cells was equally inhibited yet FTI-2153 did not inhibit bipolar spindle formation in T-24 cells. Furthermore, inhibition of bipolar spindle formation is not sufficient to inhibit tumor cell proliferation. For example, HFF cells were resistant to FTI-2153 tumor cell growth inhibition, yet FTI-2153 inhibited bipolar spindle formation in these cells.

The results of this study suggest that at the prophase/metaphase transition during mitosis, certain farnesylated proteins are required for bipolar spindle formation. The centromere-associated kinetochore protein, CENP-E has been suggested as a candidate farnesylated protein by Ashar *et al*.[22] However, we[21] and Ashar *et al*.[22] have shown that the two structurally distinct FTIs, FTI-2153 and SCH 66366 do not affect the localization of CENP-E to the kinetochore. Furthermore, inhibition of CENP-E function either by microinjecting an antibody against CENP-E or by deleting the CENP-E C-terminus (containing the CAAX sequence) does not result in inhibition of bipolar spindle formation,[23–25] but rather in inhibition of chromosome alignment at the metaphase plate. Taken together, our results and those of Schaar *et al*.[23] suggest that disruption of CENP-E function does not mediate FTI-2153 inhibition of bipolar spindle formation. To date, the mechanism by which FTIs inhibit bipolar spindle formation and accumulate cells in prometaphase is not known. Either a farnesylated protein is directly involved in centrosome separation and bipolar spindle formation, or a farnesylated protein indirectly regulates these processes. A possible candidate is the kinesin-related protein Eg5. Indeed, it has been shown that disruption of Eg5 resulted in cell cycle arrest at mitosis with unseparated spindle poles that were surrounded by a ring

of chromosomes.[26,27] Furthermore, Eg5 has 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.[28] However, Eg5 does not contain the farnesylation consensus CAAX motif. Thus, proteins that interact with Eg5 or those involved in its regulation are possible candidates for FTIs.

The inhibition of bipolar spindle formation in the primary HFF cells is intriguing and suggests that farnesylation is required for spindle formation in normal cells. Furthermore, the lack of inhibition of bipolar spindle formation by FTI-2153 in T-24 and NIH3T3 cells is also of interest. On treatment with FTIs, T-24 cells have been shown to accumulate in the G1 phase of the cell division cycle.[29] In these cells a farnesylated protein, possibly mutated H-Ras, is most likely required for the G1/S transition and its inhibition results in G1 arrest and therefore inhibition of progression to mitosis. Moreover, the absence of any FTI influence on NIH3T3 cell cycle distribution[29] may also explain the lack of inhibition of bipolar spindle formation. Furthermore, the fact that inhibition of protein farnesylation in NIH3T3 does not result in prometaphase accumulation suggest that establishment of spindle bipolarity in these cells does not require farnesylated proteins.

Taken together, our results demonstrate that the ability of FTIs to inhibit bipolar spindle formation and to accumulate cells in prometaphase during mitosis is not dependent on malignant transformation, or Ras and p53 mutation status. Our data also indicate that, depending on the cell lines, farnesylated proteins are critical at different stages of cell cycle progression and consequently FTIs could inhibit cell growth by mechanisms that involve disruption of cell cycle phases, such as G1 and G2/M.

Materials and Methods

Cell culture

OVCAR3 and NIH3T3 cells were maintained in Dulbecco's modified Eagle's medium, HFF and HT1080 cells were maintained in minimum essential medium, A-549 cells were maintained in Kaighn's F12 medium and Calu-1 and T-24 cells were maintained in McCoy's medium. All media were adjusted to contain 1.5 g/l sodium bicarbonate and were supplemented with 10% fetal bovine serum. All cells were grown in a humidified incubator at 37°C and 10% CO_2.

Flow Cytometry

Cells were plated to subconfluency on 100 mm^2 plates in order to obtain 5×10^5 to 1×10^6 cells for DNA analysis. Asynchronous cells were treated with either vehicle (DMSO) or 15 μM FTI-2153 for 72 h. Cells were then harvested with trypsin (0.05%)/EDTA (0.53 mM), washed twice 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 PI/Triton X-100 staining solution (0.1% (v/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 performed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) and ModFit *LT* 2.0 (Verity Software House, Topsham, ME, USA).

Immunocytochemistry

Cells were grown on 2-well Lab-Tek II chamber slides (Nunc, Inc., Naperville, IL, USA) to subconfluency and treated as described above. The cells were fixed in 4% paraformaldehyde (diluted with PBS from 16% stock (Electron Microscopy Sciences, Fort Washington, PA, USA)) 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% BSA in PBS for 1 h and FITC-conjugated goat anti-mouse (Sigma F9006) diluted in 0.1% Tween-20, 1% BSA in PBS for 25 min in the dark. After final washes, slides were mounted with VECTASHIELD® mounting medium with DAPI (Vector Laboratories, Inc., Burlingame, CA, USA) and viewed at $\times400$ magnification with an Orthoplan 2 fluorescent microscope (Leitz) and SmartCapture VP software (Digital Scientific).

MTT assay

Cell growth was measured by MTT [3-(4,5-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.[30] Briefly, cells were seeded at $1-4\times10^3$ cells per well in 96-well culture plates in quadruplicate. The following day, the medium was changed to DMEM supplemented with 10% FBS containing increasing concentrations of FTI-2153 or DMSO as vehicle. Cells were treated once more with the drugs in the next 48 h. Subsequently, the medium was removed and the cells were incubated with 100 μl of DMEM containing 10% FBS and 0.25 mg/ml MTT (Sigma) at 37°C for 3 h, followed by solubilization with DMSO for 10 min. The absorbance of each well was measured with a microplate reader at 570 nm. The number of viable cells is proportional to the absorbance.

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