Comparison of Potential Markers of Farnesyltransferase Inhibition[1]

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ABSTRACT

Farnesyltransferase inhibitors (FTIs) were developed to target abnormal signaling pathways that are commonly activated in neoplastic cells. Five FTIs have recently undergone Phase I testing; and two are currently in Phase II clinical trials. As part of the development of these agents, there has been interest in determining their cellular effects in the clinical setting. Several approaches have been proposed, including measurement of FT enzymatic activity, evaluation of the processing of FT polypeptide substrates, and assessment of the accumulation of p21^{waf1}. In the present study, a number of these assays have been compared in four cultured human neoplastic cell lines of different histology (A549, HCT116, BxPC-3, and MCF-7) after treatment with the nonpeptidomimetic FTI SCH66336 and the peptidomimetic inhibitor FTI-277. Immunoblotting studies failed to demonstrate a mobility shift in ras proteins or increased accumulation of p21^{waf1} after treatment with these agents. In contrast, drug-induced increases in the slower migrating, unprocessed species of the chaperone protein HDJ-2 and the intranuclear intermediate filament protein lamin A were detected in all four cell lines after treatment with either agent. Unprocessed forms of both polypeptides accumulated in noncycling as well as cycling cells. The precursor peptide that is present in prelamin A but absent from mature lamin A could be readily detected by immunohistochemistry in noncycling cells with a peptide-specific antiserum. Our results indicate that unprocessed HDJ-2 and prelamin A should be suitable markers of FT inhibition in clinical samples.

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INTRODUCTION

FT[3] catalyzes the first step in the posttranslational modification of a small number of cellular polypeptides (1, 2). Among the polypeptide substrates of FT are the ras proteins, which are mutated in ~30% of human cancers (3). As a result of early reports indicating that farnesylation was required for maturation of ras proteins, there has been extensive interest in FT as a potential target of antineoplastic therapy (4–8). To date, at least five different FTIs, SCH66336, R115777, L778123, BMS214662, and FTI-277 have entered clinical testing.

Because these compounds are among the first potential anticancer drugs that inhibit aberrantly activated signal transduction pathways in neoplastic cells, there is considerable interest in assessing their cellular and subcellular effects in the clinical setting. Conceptually, assays of FTI action could serve two purposes. In early clinical trials, these assays will be required to determine whether FT has been inhibited at drug concentrations that are achievable in the clinical setting. In later clinical trials, these assays could potentially provide an early marker of drug efficacy if a strong correlation between assay results and clinical outcome can be established. A variety of assays have been proposed for these purposes.

Because the posttranslational processing of ras provided the initial rationale for the clinical development of FTIs (7–10), many earlier studies focused on alterations in ras itself. FT inhibition results in altered mobility of H-ras on SDS-polyacrylamide gels, particularly when H-ras-transfected murine cells are examined (7, 11–14). Unfortunately, the existence of alternative prenylation pathways makes it difficult to detect altered processing of other ras isoforms after FT inhibition in many cells (15, 16). Moreover, a number of different observations have raised the possibility that other FT substrates, notably RhoB, might be involved in the antiproliferative effects of FTIs (4, 10, 17–19). These considerations have prompted investigators to search for other assays to monitor the effects of FTIs.

Conceptually, the simplest assay for FT inhibition involves preparing extracts from FTI-treated cells and measuring the remaining ability of FT to farnesylate a substrate polypeptide. In situations in which the inhibitor dissociates from the enzyme, however, these enzymatic assays are likely to underestimate the degree of FT inhibition. Moreover, these assays are difficult to implement in the setting of multi-institution clinical trials.

Other potential assays evaluate the inhibition of processing of other FT substrates. Several farnesylated polypeptides, including the chaperone protein HDJ-2 (20), the peroxisomal protein Pxf (21), and the intranuclear intermediate filament protein lamin A (22), are known to undergo mobility shifts when FT is inhibited (23, 24). In the case of lamin A, this mobility shift reflects inhibition of proteolytic processing that removes a 13-amino acid peptide from the COOH terminus of prelamin A

[3] The abbreviations used are: FT, farnesyltransferase; FTI, farnesyltransferase inhibitor.

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to yield the mature lamin (23). Because this processing is absolutely dependent on farnesylation (25), it has been suggested that the accumulation of prelamin A containing this unique COOH-terminal peptide might be a potential marker of FT inhibition (26).

In addition to these assays, it has been proposed that certain downstream events might also be used to monitor the effects of FTIs. For example, treatment with the FTI L744832 has been shown to cause increases in levels of the cyclin-dependent kinase inhibitor $p21^{waf1}$ in p53 wild-type cells (27). In addition, the activity of downstream kinases such as raf-1, MEK, or ERK1 or the phosphorylation state of MEK and ERK1 could, in principle, be used as a readout of FT inhibition (12, 28–30).

Although a wide variety of assays have been used in previous studies, they have not been compared. Moreover, it is unclear whether some of the effects reported are specific for certain agents rather than reflecting the effects of FTIs in general. In the present study, several of these different approaches have been compared in tissue culture cell lines treated with two FTIs that have entered clinical testing, the nonpeptidomimetic FTI SCH66336 (31) and the peptidomimetic inhibitor FTI-277 (12). These experiments were designed to compare various assays of FT inhibition with respect to sensitivity and potential ease of application in the clinical setting and to determine whether these assays detect inhibition of FT by agents that are actually undergoing clinical testing.

MATERIALS AND METHODS

Materials and Methods. SCH66336 (32) was a kind gift from W. R. Bishop (Schering-Plough Research Institute, Kenilworth, NJ). FTI-277 (12) and mevastatin were purchased from Calbiochem (La Jolla, CA). [^{3}H]Mevalanolactone (specific activity, 50–60 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). Plasmid pRc/CMV7SαpMEV encoding the mevalonic acid transporter was obtained from American Type Culture Collection (Manassas, VA).

Antibodies. Monoclonal antihuman lamin A (33) was a kind gift from Frank McKeon (Harvard Medical School, Boston, MA). A high-titer polyclonal serum that recognizes the COOH-terminal domain of human prelamin A was raised by immunizing rabbits with the peptide CLLGNSSPRTQSPQN coupled to keyhole limpet hemocyanin, as described by Sinensky *et al*. (26). Polyclonal rabbit anti-ras antiserum (antibody #3) was from Calbiochem (Cambridge, MA). Monoclonal anti-$p21^{waf1}$ (antibody #11) and anti-HDJ-2 were from Neomarkers (Fremont, CA). Polyclonal chicken sera that recognize lamins A and C or lamin B were raised as described previously (34). Affinity-purified peroxidase- and fluorochrome-coupled secondary antibodies were from Kirkegaard & Perry (Gaithersburg, MD).

Tissue Culture, Colony-forming Assays, and Immunoblotting. A549 human non-small cell lung cancer cells, HCT116 colon carcinoma cells, BxPC-3 pancreatic carcinoma cells, and MCF-7 breast cancer cells were obtained from American Type Culture Collection and propagated in the tissue culture media specified by the supplier. To assess the effect of SCH66336 on colony formation in A549 cells, aliquots containing 500 cells were plated in triplicate plates for each data point. After a 14–18 h incubation to allow cells to adhere, the indicated concentrations of SCH66336 (added from 1000-fold concentrated stocks prepared in DMSO) or the corresponding volume of diluent was added. Cells were incubated for 7–8 days to allow colonies to form. Alternatively, cells were incubated with SCH66336 for 24 h, washed twice in serum-free RPMI 1640, and incubated for 7 days in medium A (RPMI 1640, 5% heat-inactivated fetal bovine serum, 100 units/ml penicillin G, 100 μg/ml streptomycin, and 2 mM glutamine) in the absence of SCH66336.

To prepare whole-cell lysates for immunoblotting, replicate 100-mm dishes containing 30–40% confluent cells were treated with the indicated concentrations of SCH66336 or diluent for 22–24 h. Alternatively, A549 cells were allowed to reach confluence and maintained in this state for 7 days prior to treatment with SCH66336 for 24 h. The highest concentration of SCH66336 used in these assays, 400 nM, is at or below the trough concentration achieved in patients on prolonged schedules of SCH66336 (35). Flow cytometry (36) confirmed that >95% of the cells were in G_0-G_1 after culture under confluent conditions. At the completion of the incubation, log phase or confluent cells were washed three times with ice-cold RPMI 1640 containing 10 mM HEPES (pH 7.4) and solubilized in buffer consisting of 6 M guanidine hydrochloride, 250 mM Tris-HCl (pH 8.5 at 21°C), 10 mM EDTA, 1% (v/v) β-mercaptoethanol, and 1 mM α-phenylmethylsulfonyl fluoride (freshly added from a 100 mM stock in anhydrous isopropanol). In some experiments, SCH66336 was replaced with 20 μM FTI-277 (added from a 1000-fold concentrated stock prepared in DMSO containing 10 mM DTT), a concentration chosen because it inhibited ras farnesylation and raf activation in tissue culture in a previous study (12).

After the whole-cell lysates were dialyzed and lyophilized as described previously (37), aliquots containing 50 μg of protein [assayed by the bicinchoninic acid method (38)] were subjected to electrophoresis on SDS-polyacrylamide gels containing 8% (w/v) acrylamide (for lamins or HDJ-2) or 12% acrylamide ($p21^{waf1}$, ras), transferred to nitrocellulose, and probed with the immunological reagents described above. Antigen-antibody complexes were detected using peroxidase-coupled secondary antibodies and ECL enhanced chemiluminescence reagents.

Transfection. A549 cells (2×10^7; 50% confluent) were transfected with 40 μg of plasmid pRc/CMV7αpMEV (prepared using a plasmid isolation kit; Qiagen, Valencia, CA) by electroporation using a Bio-Rad Gene Pulser electroporator operating at 300 V, 960 μfarad and infinite resistance. Cells were cultured at low density in medium A for 24 h. Stably transfected clones were selected in 2 mg/ml G418 and isolated using cloning rings. After expansion, clones were examined for ability to accumulate [^{3}H]mevalonate during a 22–24 h incubation at 37°C. Of 25 clones examined, one with substantial mevalonate uptake was identified and named A549pMEV.

Immunohistochemistry. A549 cells grown on 20-mm glass coverslips were incubated in medium A for a minimum of 1 week after reaching confluence. Cells were then treated with 200 nM SCH66336 or diluent for 24 h as described above. At the completion of the incubation, coverslips were washed twice with ice-cold PBS and fixed in acetone for 15 min at −20°C.

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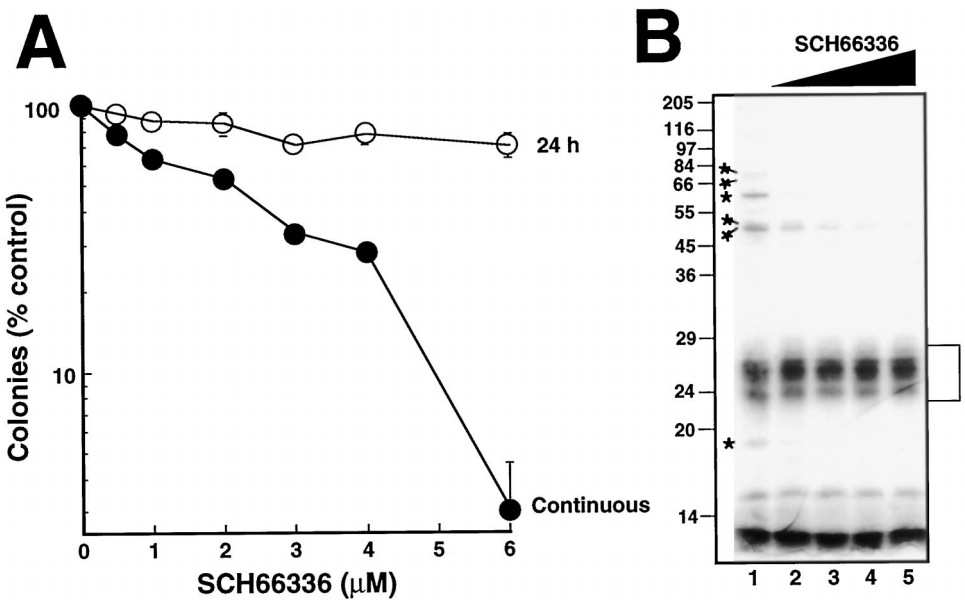

Fig. 1 Effect of SCH66336 on colony formation and protein prenylation in A549 cells. *A,* A549 cells were exposed to the indicated concentration of SCH66336 for 24 h, washed, and incubated in drug-free medium for 7 days (open circles). Alternatively, cells were exposed to SCH66336 continuously (●). *B,* A549pMEV cells were incubated for 24 h with 20 μM compactin and 1 μCi/ml [^{3}H]mevalonate in the presence of diluent (*Lane 1*) or SCH66336 at a concentration of 250 nM (*Lane 2*), 500 nM (*Lane 3*), 1000 nM (*Lane 4*), or 2000 nM (*Lane 5*). At the completion of the incubation, samples were subjected to SDS-PAGE and fluorography. *Numbers* at *left,* sizes of molecular weight markers (in thousands). *, species whose labeling decreases with SCH66336 treatment. *Bracket* at *right,* small GTP-binding proteins whose prenylation has been reported previously to be resistant to FTI treatment (11).

Samples were then rehydrated with two to three changes of PBS and blocked for a minimum of 1 h at 4°C in buffer B, which consisted of 10% (w/v) powdered milk in 150 mM NaCl, 10 mM Tris-HCl (pH 7.4 at 21°C), 100 units/ml penicillin G, 100 μg/ml streptomycin, and 1 mM sodium azide. Cells were treated with a mixture of murine monoclonal anti-lamin A (1:3000) and rabbit anti-prelamin A (1:750) in buffer B at 4°C for 12–16 h. After removal of the primary antibodies, samples were washed six times with PBS over a 20-min period, incubated for 30 min with a mixture of affinity-purified, rhodamine-conjugated antimouse IgG and fluorescein-conjugated antirabbit IgG (20 μg/ml each), washed six times with PBS over a 20-min period, mounted in Vectashield (Vector Laboratories, Burlingame, CA), sealed with clear nail polish, and examined on a Zeiss LSM 310 confocal microscope (Carl Zeiss, Inc., New York, NY).

RESULTS

SCH66336 Inhibits Protein Prenylation in A549 Cells. In initial experiments, we examined the effects of the FTI SCH66336 (31, 32) on A549 cells, a non-small cell lung cancer line containing mutated K-ras (39, 40). Inclusion of increasing concentrations of SCH66336 in the tissue culture medium resulted in a dose-dependent decrease in the number of colonies that formed after 7–8 days (Fig. 1*A*, ●). In contrast, a 24-h exposure to SCH66336 had a minimal effect on colony formation (Fig. 1*A*, ○), indicating that SCH66336 was not killing the cells under these conditions. This model system provided the opportunity to examine the effects FT inhibition without secondary changes related to cytotoxic effects *per se*.

To confirm that SCH66336 was inhibiting protein farnesylation *in situ*, the covalent modification of various polypeptides by metabolic derivatives of mevalonic acid was examined in A549 cells transfected with the mevalonate transporter (Fig. 1*B*). This analysis revealed that SCH66336 caused a dose-dependent decrease in prenylation of several different polypeptides with apparent molecular weights between M_r ~18,000 and M_r ~75,000 (Fig. 1*B*, *). The prenylation of these polypeptides was detectably decreased at 250 nM SCH66336 (Fig. 1*B*, *Lane 2*) and completely inhibited at 1–2 μM (*Lanes 4* and *5*). These results confirm that SCH66336 inhibits the prenylation of several polypeptide species in this model system. In contrast, a number of polypeptides in the M_r 20,000–30,000 region were prenylated despite the presence of high concentrations of SCH66336 (Fig. 1*B*, *bracket*). These polypeptides, which were identified previously as small GTP-binding proteins, have been reported to be prenylated in the presence of other FTIs as well (11).

Examination of Altered Protein Mobility after FTI Treatment. In additional experiments, whole-cell lysates prepared from A549 cells after treatment with varying concentrations of SCH66336 were subjected to SDS-PAGE, followed by immunoblotting with reagents raised against polypeptides that are FT substrates. When blots were probed with a serum that recognizes all ras species, altered mobility of the predominant ras species in this cell line was not detectable (Fig. 2*A*, *top panel*). In the same samples, only a small percentage of the farnesylated nuclear protein lamin B (41) demonstrated altered mobility (Fig. 2*B*, *top panel*). In contrast, increased amounts of a slower migrating species of HDJ-2 were observed after

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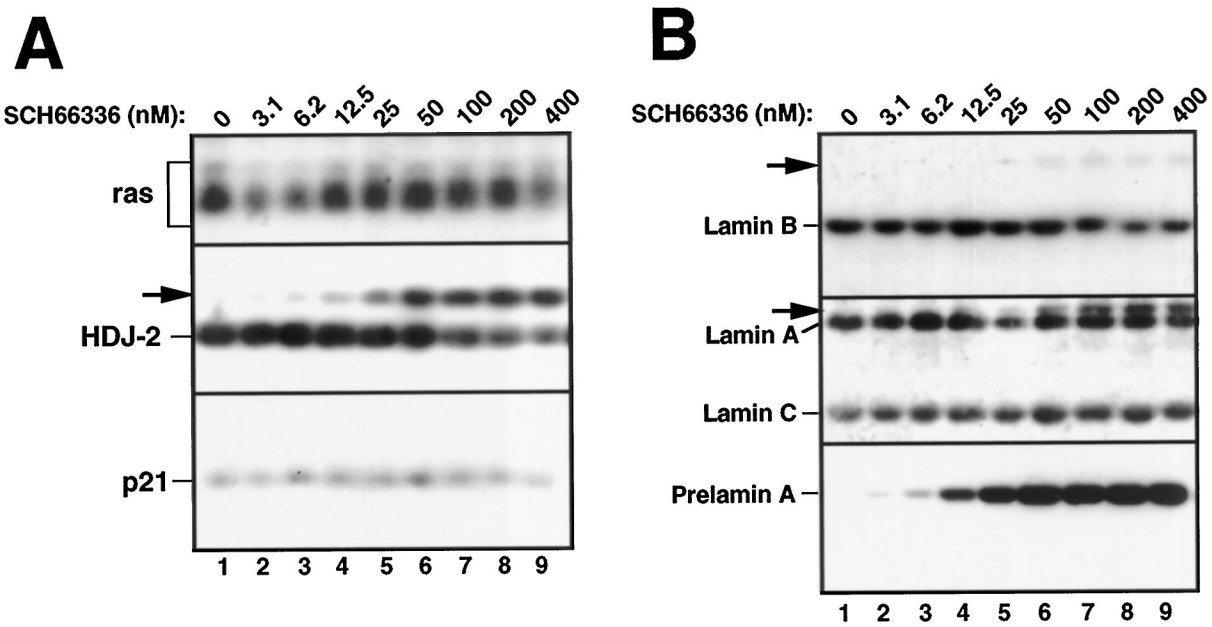

Fig. 2 Effect of SCH66336 on levels and migration of various polypeptides that have been proposed as markers of FT inhibition. *A,* A549 cells incubated for 24 h with the indicated concentration of SCH66336 were subjected to SDS-PAGE, followed by blotting with probes that recognize all ras isoforms (*top*), HDJ-2 (*middle*), or $p21^{waf1}$ (*bottom*). *B,* A549 cells treated for 24 h with the indicated concentration of SCH66336 were blotted with antisera that recognize lamin B (*top*), lamins A and C (*middle*), or the peptide LLGNSSPRTQSPQN at the COOH terminus of prelamin A (*bottom*). *Arrows,* slower migrating species of HDJ-2, lamin B, and lamin A in the respective panels.

SCH66336 treatment (Fig. 2*A, second panel*). This slower migrating species was readily detectable at drug concentrations as low as 6.25 nM and amounted to as much as half of the total HDJ-2 at 400 nM SCH66336.

When these same blots were probed with antibodies to the nuclear protein lamin A, a slower migrating species that was identified previously as prelamin A (23, 26) was seen at SCH66336 concentrations as low as 25 nM (Fig. 2*B, second panel*). Probing of the same blot with an antiserum that specifically recognizes the precursor polypeptide at the COOH terminus of prelamin A (26) revealed that the inhibition of prelamin A processing could be detected at SCH66336 concentrations as low as 6.25 nM (Fig. 2*B, bottom panel*).

To look for downstream markers that might be useful for examining FT inhibition, blots were also probed with antibodies to $p21^{waf1}$. Although another FTI has been reported to increase $p21^{waf1}$ levels in certain cells (27), levels of this polypeptide did not increase in A549 cells after treatment with SCH66336 (Fig. 2*A, bottom panel*).

To rule out the possibility that these results were unique to A549 cells, three additional cell lines were treated with 200 nM SCH66336 and subjected to the same analysis. The cell lines chosen for this analysis were the pancreatic carcinoma line BxPC-3, which has wild-type ras (42) and displays an IC_{50} of 200 nM upon continuous exposure to SCH66336 in clonogenic assays;[4] the breast carcinoma line MCF7, which has wild-type ras (43) and displays an IC_{50} of 50 nM upon continuous exposure to SCH66336 in clonogenic assays;[4] and the colon carcinoma line HCT116, which has a mutant K-ras (42) and displays an IC_{50} of 100 nM upon continuous exposure to SCH66336 in clonogenic assays.[4] Treatment for 24 h with 200 nM SCH66336 again failed to demonstrate any alteration in the mobility of detectable ras proteins by immunoblotting (Fig. 3, *top panel, Lane 2*). In contrast, increased levels of the slower migrating HDJ-2 species were readily detectable in all four cell lines after FTI treatment (Fig. 3, *second panel, Lane 2*). Moreover, each of the FTI-treated cell lines contained a slower migrating species of lamin A that reacted with the prelamin A antiserum (Fig. 3, *third panel, Lane 2*). As was the case in A549 cells, $p21^{waf1}$ failed to reproducibly increase in the other three cell lines after SCH66336 treatment (Fig. 3, *bottom panel, Lane 2*).

To rule out the possibility that the observed results were unique to the nonpeptidomimetic FTI SCH66336, all four cell lines were also treated with the peptidomimetic inhibitor FTI-277. Results of this analysis (Fig. 3, *Lanes 3*) demonstrated that FTI-277 altered processing of HDJ-2 and prelamin A. In contrast, altered migration of ras and altered levels of p21 were not observed. Identical results were also obtained when the FTI R115777 was substituted for SCH66336 (data not shown).

Effects of FT Inhibition on Noncycling Cells. Because the vast majority of cells in clinical tumor specimens are not cycling at any point in time, we evaluated these assays in noncycling A549 cells. Seven days after reaching confluence, the vast majority of these cells were in G_0-G_1 (Fig. 4*A, bottom panel*). In the confluence-arrested cells, increased levels of the slower migrating species of HDJ-2 and the appearance of pre-

[4] J. N. Davis and A. A. Adjei, unpublished observations.

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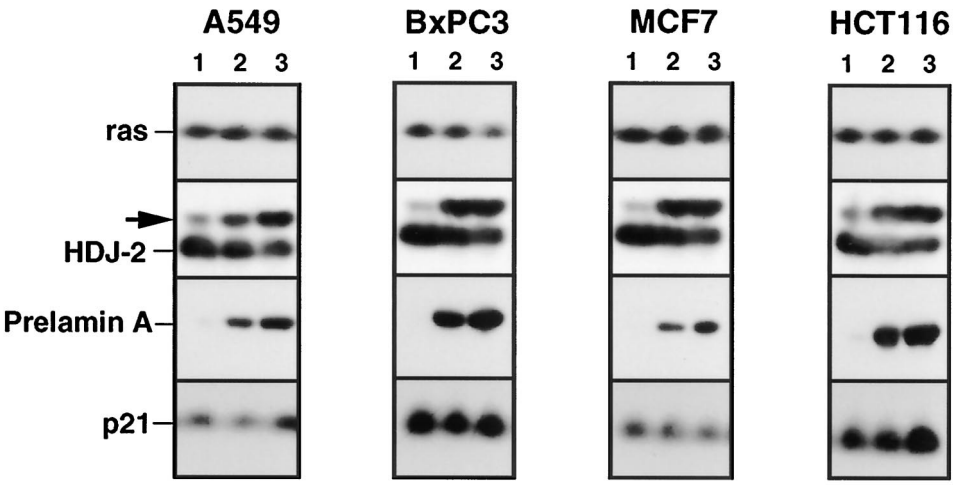

Fig. 3 Evaluation of assays in various cell types. A549, HCT116, BxPC-3, and MCF-7 cells were treated for 24 h with diluent (*Lane 1*), 200 nM SCH66336 (*Lane 2*), or 20 μM FTI-277 (*Lane 3*). At the completion of the incubation, whole-cell lysates were subjected to SDS-PAGE, followed by transfer to nitrocellulose and blotting with reagents that recognize all ras isoforms (*top panel*), HDJ-2 (*second panel*), prelamin A (*third panel*), or $p21^{waf1}$ (*bottom panel*). Note that both drugs induced increases in the slower migrating species of HDJ-2 and prelamin A in all four cell lines.

lamin A were readily demonstrated after SCH66336 treatment (Fig. 4*A*), although the signals were slightly decreased in noncycling cells compared with cycling A549 cells examined on the same blot.

Immunohistochemical Staining for Prelamin A. Of the markers examined above, prelamin A is unique in containing a large peptide that is absent from the processed protein. It has been suggested previously that the presence of prelamin A can be detected by immunohistochemistry after treatment of log phase cells with the benzodiazepine-based FTI BZA-5*B* (26). To investigate the potential usefulness of this assay with other FTIs, as well as determine the suitability of this approach for assessing FTI action in noncycling cells, confluence-arrested A549 cells were treated with SCH66336 and then stained for prelamin A in the absence or presence of the peptide used to raise the serum. Results of this analysis revealed that prelamin A was at the limit of detection in untreated cells (Fig. 4*B, top row*) but became readily detectable in all of the cells by immunofluorescence after treatment with SCH66336 (Fig. 4*B, middle row*). The immunizing peptide prevented labeling with this serum (Fig. 4*B, bottom row*), demonstrating specificity of this reaction. When combined with the immunoblotting results in Fig. 3, these observations not only confirm that multiple FTIs inhibit prelamin A processing but also indicate that this inhibition can be detected in noncycling cells by immunoblotting (Fig. 4*A*) or immunohistochemistry (Fig. 4*B*).

DISCUSSION

The present study compared the performance of several different assays that have been proposed for monitoring FT inhibition in the clinical setting. This study used the nonpeptidomimetic FTI SCH66336 and the peptidomimetic inhibitor FTI-277, both of which have entered clinical trials. Results of these assays were compared in four separate cell lines and in log-phase *versus* confluent, noncycling cells. These results have potential implications for monitoring FT inhibition in the clinical setting.

Because the FTIs are among the first inhibitors of signal transduction to be widely tested in the clinic, there is considerable interest in assessing their effects in clinical material. An ideal marker for such studies would be one that is highly sensitive to inhibition of the targeted pathway, selective for inhibition of that pathway, and assayable in a wide variety of tumor types as well as potentially in surrogate normal tissues. Other features that would be desirable in a marker assay would be ease of performance and the ability to analyze samples in batches rather than individually. As signal transduction inhibitors are tested clinically, such assays will be particularly important in differentiating between compounds that successfully target the intended pathway but do not have clinical activity *versus* compounds that fail to target the intended pathway at concentrations where toxicity prohibits dose escalation. Distinguishing between these alternatives will be particularly important in clinical trials that do not have the classical end point of tumor shrinkage as well as in chemoprevention and adjuvant chemotherapy trials. In light of the potential importance of these types of studies, we have compared several of the assays that have been proposed for the assessment of FT inhibition in clinical material.

One potential approach would be to assay FT enzymatic activity in cell extracts after drug exposure. This approach suffers from the potential problem that reversible inhibitors such as SCH66336 and FTI-277 might dissociate from FT during cell purification and fractionation, thereby leading to underestimation of the degree of inhibition achieved *in situ*. In addition, current assays of FT activity in certain cell types are subject to interference by endogenous inhibitors.[5] As a result, some investigators have resorted to partial purification of the enzyme prior to assaying its activity (44), an approach that does not appear feasible in the context of multicenter clinical trials.

Immunoblotting and immunohistochemistry approaches appear to be better suited for application in the clinical setting. FTIs were initially developed to inhibit the posttranslational processing of the ras oncoproteins (4, 5, 45). Several earlier

[5] D. End, personal communication.

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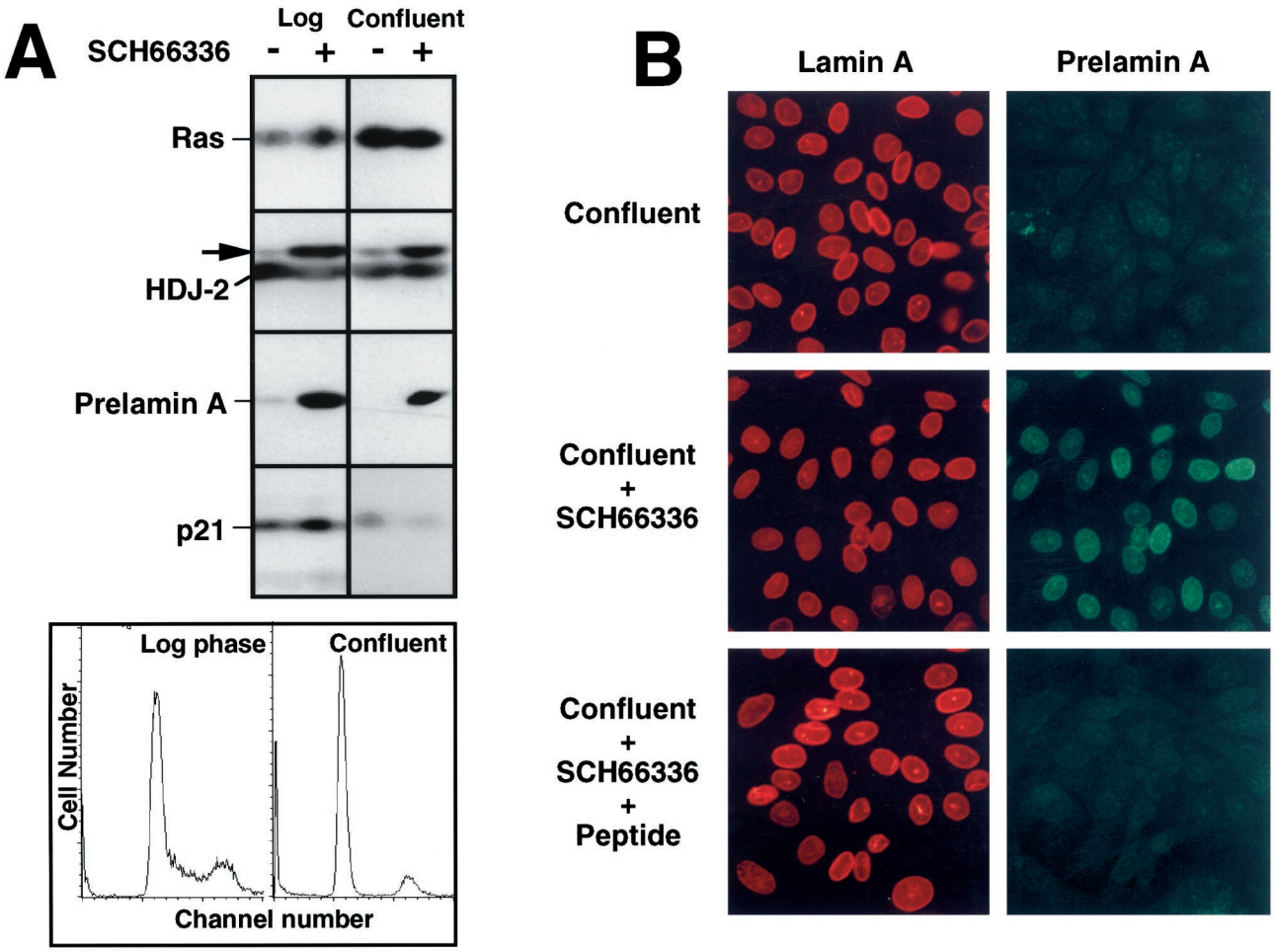

Fig. 4 Changes in HDJ-2 and prelamin A in noncycling cells. *A,* log phase or confluence-arrested A549 cells were treated with diluent or 200 nM SCH66336 for 24 h as indicated. Whole-cell lysates were subjected to SDS-PAGE, followed by immunoblotting with the indicated probe. *Bottom,* DNA histograms of log phase and confluence-arrested A549 cells. *B,* evaluation of prelamin A processing in noncycling cells. One week after reaching confluence, A549 cells were incubated for 24 h with diluent (*top row*) or 200 nM SCH66336 (*middle* and *bottom rows*). After the cells were fixed in acetone, double label indirect immunofluorescence was performed using monoclonal anti-lamin A (*left*) and polyclonal antiserum that recognizes the precursor peptide at the COOH terminus of lamin A (*right*). The incubation with primary antibodies was performed in the absence (*top two rows*) or presence (*bottom row*) of 10 μg/ml CLLGNSSPRTQSPQN, the peptide that was used to raise the prelamin A-specific serum. In each row, the same field was imaged using excitation wavelengths of 568 nm (*left*) or 488 nm (*right*).

studies demonstrated that FTI treatment altered mobility of H-ras in transfected murine cells. Nonetheless, immunoblotting experiments failed to demonstrate altered migration of endogenous ras polypeptides in a number of different cell lines after treatment with SCH66336 or FTI-277 (Figs. 2 and 3). There are several potential explanations for this result. It is possible that the predominant ras species in these cell lines are isoforms of K-ras, which has been shown to be more resistant to FTIs than H-ras (46). We note, however, that prelamin A farnesylation is also more resistant to FTIs than H-ras (47), yet we could readily detect inhibition of prelamin A processing in each of the cell lines. Alternatively, it is possible that geranylgeranylation of ras polypeptides in FTI-treated human cells (16) leads to processed ras species that have unaltered mobility on SDS-polyacrylamide gels. In either case, it is possible that immunoprecipitation of H-ras, followed by Western blotting (an approach necessitated by low levels of endogenous H-ras in these cells and many other tissue types), might demonstrate an effect of the FTIs on migration of H-ras. However, it is difficult to envision that immunoprecipitation followed by immunoblotting would be practical for routine performance in the clinical setting.

Recent studies have suggested that RhoB might also be a critical target for FTIs (4, 10, 17–19). These studies have examined epitope-tagged RhoB that is expressed after transfection. In our hands, experiments similar to those in Fig. 2 have revealed that endogenous rho species are below the limit of detection with commercially available monoclonal antibodies,[4] making it difficult to use this polypeptide as a marker of FTI action in the clinical setting. Accordingly, we focused on other potential markers of FT inhibition.

Consistent with recent results published in abstract form (24), the prenylated protein HDJ-2 demonstrated a shift in

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mobility upon treatment with SCH66336 (Figs. 2 and 3). The appearance of the slower migrating species was detectable at concentrations of SCH66336 as low as 6.25 nM (Fig. 2*A*). In addition, a similar mobility shift was observed in noncycling cells (Fig. 4*A*), indicating the potential usefulness of this assay in clinical tumor specimens.

Work from several laboratories demonstrated previously that lamin B and prelamin A are farnesylated polypeptides (23, 41). In our studies, only a small amount of lamin B displayed a mobility shift in the presence of SCH66336 (Fig. 2*B*). In contrast, as much as half of the lamin A demonstrated a mobility shift after the same treatment (Fig. 2*B*). Further analysis revealed that the processing of prelamin A was extensively inhibited in all cell lines examined (Figs. 2*B* and 3). Accumulation of prelamin A above low basal levels was detectable at SCH66336 concentrations as low as 6.25 nM and increased in a dose-dependent manner (Fig. 2*B*). Because prelamin A is ordinarily present in limited amounts within the cell, the assay for prelamin A was suitable for immunoblotting (Figs. 2–4) or immunohistochemistry (Fig. 4*B*), either of which should be potentially useful in the clinical setting. The widespread use of immunohistochemistry for other purposes (*e.g.*, assessment of estrogen and progesterone receptors, HER2-neu status, and cytokeratin expression) suggests that the immunohistochemical assay for prelamin A might be particularly useful.

Finally, as a potential example of polypeptide changes downstream from ras, we examined the up-regulation of $p21^{waf1}$. Although the FTI L744832 has been reported to induce accumulation of $p21^{waf1}$ in a p53-dependent manner, we could not detect increased $p21^{waf1}$ in any of the four cell lines after treatment with SCH66336 (Figs. 2 and 3) or FTI-277 (Fig. 3). In this context, it is important to note that A549, MCF-7, and HCT116 cells all have wild-type p53. These observations not only raise the possibility that up-regulation of $p21^{waf1}$ after treatment with L744832 might be a drug- or cell line-specific effect but also highlight the importance of examining potential markers of FTI action with the compounds that are actually undergoing clinical testing.

To rule out the possibility that some of the results described in the present work might be unique to SCH66336 and FTI-277, experiments depicted in Figs. 2–4 were repeated using R115777, another FTI that is in Phase II clinical trials (10). All of the results presented above were readily reproduced with R115777.[4]

In summary, the current findings identify two different assays that appear to be sensitive and widely applicable markers of FT inhibition. HDJ-2 is present in a variety of tumor cell lines and undergoes a readily detectable mobility shift upon treatment with FTIs. Lamin A likewise undergoes a mobility shift in multiple cell lines after FTI treatment. The mobility shift of HDJ-2 and the accumulation of prelamin A are detectable at similar drug concentrations. The retention of a unique prepeptide in prelamin A after FTI treatment makes this polypeptide amenable to assay by both immunoblotting and immunohistochemistry. Because the effects on HDJ-2 and lamin A are observed in noncycling as well as cycling cells, one or both of these assays should be suitable for detecting inhibition of FT in the clinical setting.

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