Nonpeptidomimetic Farnesyltransferase Inhibitor RPR-115135 Increases Cytotoxicity of 5-Fluorouracil: Role of p53

PATRIZIA RUSSO, CRISTINA OTTOBONI, DAVIDE MALACARNE, ALESSANDRA CRIPPA, JEAN-FRANCOIS RIOU, and PATRICK M. O’CONNOR

Laboratory of Molecular Pharmacology, Division Basic Sciences, National Cancer Institute, National Institutes of Health, Bethesda, Maryland (P.R., C.O., P.M.O.); Laboratory Experimental Oncology, Molecular Pathology Section, National Institute for Research on Cancer, Genova, Italy (P.R., C.O., D.M., A.C.); and Anticancer Research Program, Centre de Recherche Rhone-Pouilenc Rorer-Aventis Pharma, Vitry sur Seine, France (J.-F.R.)

Received July 13, 2001; accepted August 13, 2001 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

A new nonpeptidic farnesyltransferase inhibitor, RPR-115135, in combination with 5-fluorouracil (5-FU) was studied in an isogenic cell line model system consisting of human colon cancer HCT-116 cells. HCT-116 cells were transfected with an empty control pCMV vector and with a dominant-negative mutated p53 transgene (248R/W). We found that, relative to control transfectants, there was a slight tendency for the p53 inactivated cells to be less sensitive to 5-FU after 6 days of continuous treatment. Simultaneous administration of RPR-115135 and 5-FU, at equitoxic concentrations, resulted in an enhancement of 5-FU cytotoxicity, especially in the CMV-2 clone. Growth inhibition could be accounted for on the basis of a specific cell cycle arrest phenotype (G2-M arrest in CMV-2 and S arrest in mutated clones), as assayed by flow cytometry. The combination RPR-115135 + 5-FU increases apoptotic events only in the CMV-2 clone.

Although about 50% of patients with colorectal cancer are cured by surgery alone, this disease is a leading cause of morbidity and mortality. For decades, single agent 5-FU has been the main drug used in the treatment of metastatic colorectal cancer. However, the drug’s real efficacy and optimal schedule are still being discussed. The response rate to intravenous bolus 5-FU was evaluated in a meta-analysis that included 10 trials comparing bolus 5-FU with FU modulated by leucovorin (FA). The response rate was 11% for bolus 5-FU, including 3% complete response. Although the clinical results of FA have shown increased response rates (23%), the impact of this treatment on survival was not documented and overall median survival was 11.5 and 11 months, respectively. Despite no survival benefit in advanced disease, it has been demonstrated that FA used in the adjuvant setting significantly reduces mortality compared with 5-FU alone (35 versus 22%) (The ACCMAP, 1992; Midgley and Kerr, 1998; IMPACT, 1995; Sobrero et al., 2000).

Recent insights into the biology of colon cancer have spurred development of new drugs [i.e., new folate-based thymidilate synthetase (TS) inhibitors (tomudex, AG337, or LY231514), inhibitors of glycinamide ribonucleotide formyltransferase (AG2034, lemetrexol), topoisomerase I inhibitors (camptocecin-11), or farnesyltransferase inhibitors (R11577, SCH66336)] (Rustum and Cao, 1999; Schmoll et al., 1999). The wealth of new knowledge concerning the molecular and biochemical pathways required for neoplastic transformation has provided important insights into the clinical behavior of colorectal cancer. It is now widely accepted that the multistep carcinogenic process that is involved in colon cancer is driven by mutational events that ultimately give the cancer cells a growth advantage (Midgley and Kerr, 1999).

Alterations in genes related to cell cycle regulation may have profound impact on the efficacy of chemotherapy. It has been suggested that modifications of p53 that influence a cell’s tendency to apoptosis may play a significant role in modifying response to radiation and chemotherapy. The cytotoxic effects of 5-FU depend on the induction of cell apoptosis. 5-FU cytotoxicity is determined by either thymidine deprivation (inhibition of TS) or by RNA incorporation. Data (Pritchard et al., 1997, 1998; Bunz et al., 1999) strongly support the hypothesis that cell death in intestinal epithelia

ABBREVIATIONS: 5-FU, 5-fluorouracil; FA, leucovorin; TS, thymidilate synthetase; FTase, farnesyltransferase; CI, combination index.
requires 5-FU metabolites to be incorporated into RNA. Cell death therefore occurs by apoptosis and is p53-dependent.

Cytotoxicity of 5-FU in cell lines of the National Cancer Institute anticancer drug screening program correlated with their p53 status (O'Connor et al., 1997). Disruption in p53, by targeted homologous recombination, rendered human colon cancer cell lines strikingly resistant to the effects of 5-FU. The effects on 5-FU sensitivity were observed both in vitro and in vivo, were independent of p21waf-1, and appeared to be the result of alterations in RNA, rather than DNA, metabolism (Bunz et al., 1999). Although significant experimental evidence in laboratory models has accumulated to demonstrate that p53 status influences chemotherapy, a strong relationship with p53 status and response to 5-FU in colon cancer patients has not been demonstrated (Aschele et al., 1997; Ahnen et al., 1998). However, Ahnen et al. (1998) have suggested that patients with stage III colon cancer with wild-type K-Ras and wild-type p53 benefit from adjuvant 5-FU plus levamisole therapy.

Of the many signal transduction mechanisms that are emerging as potential targets for future cancer drugs, the preylation of Ras family proteins is receiving particular attention from both pharmaceutical companies and academic groups (Gibbs, 2000; Giraud et al., 2000; Hill et al., 2000; Reuter et al., 2000). Studies have shown that farnesylation of Ras is the obligatory first step in a series of post-translational modifications that lead to membrane association, allowing the switch between an inactive Ras-GDP-bound form to an active Ras-GTP-bound form. Ras-GTP acts as a molecular switch that turns on downstream effectors (Katz and McCormick, 1997). FTase is responsible for catalyzing farnesylation of several cellular proteins by transfer of a C-15 farnesyl moiety from farnesyl pyrophosphate. Previous studies by our group have been demonstrated (Russo et al., 1998, 1999) that a nonpeptidomimetic inhibitor, namely, RPR-115135, recently developed by Aventis Pharma (Center de Recherches de Vitry Alfortville, France) was able to inhibit the cell growth in an isogenic cell system. The isogenic cell system consisted of human colon cancer HCT-116 cells. The present study was undertaken to investigate the possible effects of RPR-115135 used in combination with 5-FU in the above-mentioned isogenic cell system.

Materials and Methods

Chemical Treatments. RPR-115135 (C31H29NO4, mol. wt. 479.58) is produced by Aventis Pharma. It was prepared as a 1 mM stock solution in dimethyl sulfoxide and aliquots were stored at −20°C until needed. 5-FU was purchased by Sigma Chemical (St. Louis, MO).

Cell Culture. Human colon cancer cell line HCT-116 was grown in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 5% heat inactivated fetal bovine serum and 2 mM glutamine. Cells transfected with either empty control vector (pcMV) or vector containing a dominant-negative mutant p53 transgene (248R/W) (cloned into a pcMV plasmid) to inhibit p53 function were grown in the same medium. The generation and characterization of the HCT-116 transfectants have been described previously (Fan et al., 1997, 1998). Different clones (CMV (2 or 4) or Mu-p53 (2 or 4)) were examined. HCT-116 cells harbor a K-Ras mutation at residue 13 that disrupts GTPase activity, locking the Ras protein in its active form (Koo et al., 1996). Cell counts were determined using a Beckman Coulter counter with Channelizer attachment to monitor cell size (Beckman Coulter, Inc., Fullerton, CA). Cell membranes integrity was determined by trypan blue dye exclusion assay.

Cell Cytotoxicity. Cells were plated in log phase into 96-multiwell plates (250 cells/well) with 190 μl of complete medium for 24 h and then treated with various concentrations (0.1–10 μM) of RPR-115135 (10 μl) for 2 or 6 days. At the end of the incubation time (2 or 6 days), 40 μl of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2H-tetrazolium inner salt (CellTiter 96 AQueous, one Solution Cell Proliferation Assay; Promega, Madison, WI) was added for 2 h and then adsorbence was read at 490 nm with a 96-well plate reader. The IC_{50, 6 days}, IC_{50, 2 days}, IC_{50, 0 days} was calculated, respectively, as the drug concentration that inhibits cell growth to 12.5, 25, 50, or 75% of the control cells.

Flow Cytometry. Cells were plated in log phase in T75 flasks (2700 cells/cm²) in complete medium for 24 h, treated over 6 days with 5-FU in combination with RPR-115135, and then counted before flow cytometry. Samples were prepared for flow cytometry essentially as described previously (O'Connor et al., 1997). Briefly, cells were washed with 1× phosphate-buffered saline, pH 7.4, and then fixed with ice-cold 70% ethanol. Samples were washed with 1× phosphate-buffered saline and stained with 6 μg/ml propidium iodide (Sigma Chemical) containing 2 μg/ml RNase (Sigma Chemical) for 30 min at 37°C. Cell cycle analysis was performed using a BD Biosciences fluorescence-activated cell analyzer and Cell Quest, version 1.2, software (BD Biosciences, San Jose, CA). For each sample at least 15,000 cells were analyzed and quantitation of the cell cycle distribution was performed using ModFit LT, version 1.01, software (Verity Software House, Topsham, ME).

DNA Secondary Fragmentation Assay. Apoptosis-associated DNA fragmentation was analyzed by filter-binding assay as previously described (Bertrand and Pommier, 1996). A filter-binding assay was performed under nondeproteinizing conditions using protein-adsorbing filters (vinyl/acrylic copolymers filters, Metrical membrane, 0.8-μm pore size, 25 mm in diameter; Gelman Instrument Co., Ann Arbor, MI) according to Debernardis et al. (1997). Prelabeled cells (0.5 × 10^6) with 0.02 μCi/ml [3H]thymidine were loaded onto polynvinyl chloride filters and washed with 5 ml of Hanks' balanced salt solution. Cells were then lysed with 5 ml of solution containing 0.2% sodium sarkosyl, 2 M NaCl, 0.04 M EDTA, pH 10.0. After the lysis solution had dripped through by gravity, it was washed from the filter with 5 ml of 0.02 M EDTA, pH 10.0. Filters were then processed as in the case of alkaline elution (Bertrand and Pommier, 1996). Radioactivity was counted by liquid scintillation spectrophotometry in each fraction (loading fraction, wash, lysing EDTA washes relative to total intracellular [3H]-labeled DNA. Data are calculated as the percentage of DNA fragmented in treated cells compared with DNA fragmented in control, untreated cells (background) using the formula [(F F)/(F F)] × 100, where F and F represent DNA fragmentation in treated and control cells, respectively.

Results

Growth Inhibition. Previous work has shown the status of p53 gene and pathway in the human colon cancer HCT-116 isogenic system used in the present studies (Fan et al., 1997, 1998). After γ-irradiation, only parental and control transfectant CMV cells (two clones) but not Mu-p53 cells (two clones) were able to arrest in the G1 phase of the cell cycle and accumulate p53 or p21^{CIP1-waf-1} proteins. These experiments clearly showed that the functions of p53 in the transfected p53 mutated cells are disrupted (Fan et al., 1997, 1998). In spite of the different status of p53, in the different cell clones, these cells are not prone to apoptosis induced by γ-irradiation (Fan et al., 1997, 1998).
There is debate concerning the predominant mechanism of cytotoxicity associated with exposure to low concentrations of 5-FU. In HCT-116 cells RNA-directed effects have been predominant with prolonged duration of 5-FU exposure (over 7 days), whereas DNA-directed effects have been important during short-term exposure (6–48 h) (Ren et al., 1997); for this reason, we have initially investigated the sensitivity to 5-FU, over 2- or 6-day exposure, in HCT-116 parental cells, in two clones transfected with the empty control vector pCMV (CMV-2/CMV-4) or in two clones transfected with a negative dominant p53 (Mu-p53-2/Mu-p53-4). Upon 2 days of treatment with 5-FU all cell lines were growth inhibited, with no difference in terms of IC50 values (Table 1; p > 0.05, Student’s t test).

After 6 days of continuous treatment, the 5-FU sensitivity was slightly higher (about 4–5 times) in the p53 active cells (parental, CMV-2 or CMV-4 clone) than in the inactive ones (Mu-p53-2 or Mu-p53-4 clone) (Table 1; p < 0.002, Student’s t test). These data are in agreement with other observations (Pritchard et al., 1997, 1998; Ren et al., 1997; Bunz et al., 1999).

RPR-115135 was inactive when cells were treated for 2 days (data not shown). When cells were treated simultaneously with RPR-115135 (range 0.1–10 μM) and with different concentrations of 5-FU (range 1–100 μM) for 2 days, no potentiating effect was observed in both two clones (data not shown). After 6 days of treatment with RPR-115135 all cell clones were growth inhibited, with similar IC50 values, as previously reported (Russo et al., 1998, 1999).

When RPR-115135 was added to the medium simultaneously with 5-FU, at equitoxic concentrations for 6 days continuous exposure, a substantial growth inhibition (statistically significant, p < 0.002, Student’s t test) was observed in the CMV-2 clone. The IC50 of the combination was 0.6 μM (1.2 μM 5-FU alone). A small potentiation was observed in the Mu-p53-2 clone [IC50 of the combination was 1.9 μM versus 2.6 μM for 5-FU alone (statistically not significant, p = 0.05, Student’s t test)].

**Time Course, Cell Cycle Experiments, and Induction of Secondary DNA Fragmentation.** Time course experiments were performed treating CMV-2 or Mu-p53-2 clone to different concentrations of 5-FU alone, or RPR-115135 alone, or in combination at equitoxic concentrations (range IC12.5–IC50). These experiments confirmed that CMV-2 clone was more sensitive than the Mu-p53-2 clone at 5-FU (Fig. 1, A and B). When subtoxic concentrations of RPR-115135 and 5-FU (Fig. 1A) were given together a greater than additive effect in growth inhibitory activity was observed in CMV-2 clone (p < 0.01; Fig. 1A). This observation was supported by CI = 0.01 (where CI < 0.3, strong synergism; CI = 1, additive; and CI > 1, antagonism; Chou and Talalay, 1977, 1981). The potentiation effect was also present by increasing drug concentrations (Fig. 1, B and C). At the IC50, it was difficult to evaluate the potentiation effects because the cells’ inhibition reached the plateau level (Fig. 1C).

Because mutated cells are more resistant to 5-FU we studied the role of p53 suppressor mutation to the degree of additivity. The potentiation was apparently present, only at higher drug concentrations, in the Mu-p53-2 clone (Fig. 2, A–C). Although in the CMV-2 clone the combination 5-FU + RPR-115135 (at IC12.5 value) was greater than additive in growth inhibition (Fig. 1A), in Mu-p53-2 clone it was antagonistic with CI = 4.55 (strong antagonism, p < 0.05; Fig. 2A).

At higher concentrations (IC25 and IC50) a small effect was seen (CI = 0.815, slight synergism; Fig. 2, B and C) (Chou and Talalay, 1977, 1981).

The cell cycle was analyzed over 6 days of continuous treatment. Table 2 shows the percentage of cell survival after 6 days of continuous experiments. At concentrations of 5-FU equal to IC12.5 both clones are not growth inhibited, but at concentrations equal to IC25, CMV-2 clone was more inhibited than Mu-p53-2 clone. When the two drugs were administered simultaneously, the potentiation effect was present in both clones but it was more relevant in the CMV-2 clone.

Growth inhibition could be accounted for on the basis of a specific cell cycle arrest phenotype, as assayed by flow cytometry (Fig. 3, A and B). Treatment with RPR-115135, or with 5-FU, at concentration equal to IC12.5, did not produce any apparent alteration in the composition of the cell cycle in both two clones (Fig. 3, A and B). These data for RPR-115135 are in agreement with previous data, obtained using higher concentrations (IC50 and IC25) (Russo et al., 1998, 1999). 5-FU at a concentration equal to IC50 (Fig. 3A) in CMV-2 clone induced a strong G2-M arrest, whereas in the Mu-p53-2 clone induced an S increase (Fig. 3B). The effect observed for the combination 5-FU + RPR-115135 (at concentrations equal, respectively, to IC12.5, Values in the CMV-2 clone or to IC25, values in the Mu-p53-2 clone) was similar to 5-FU at IC50 value. The same picture was also observed in two others different clones (CMV-4 and Mu-p53-4; data not shown).

Flow cytometry in the CMV-2 clone indicated the presence of a sub-G1 population starting 2 days after treatment with 5-FU alone (IC50 value) or with the combination 5-FU + RPR-115135 (at concentrations equal, respectively, to the IC12.5 values; Fig. 4A).

In Mu-p53-2 clone the sub-G1 population was less significant (Fig. 4B). Because the above-mentioned data may suggest an induction of apoptosis, the induction of DNA secondary fragmentation (apoptosis-related) was evaluated by means of filter-binding assay. Figure 5A shows that in the CMV-2 clone a strong induction of DNA secondary fragmentation was observed only for the combination 5-FU + RPR-115135 (at the IC12.5 values, respectively). It started 1 day after treatment and was maximum after 4 days. Also 5-FU alone at the highest concentration tested (IC50) induced DNA secondary fragmentation. Figure 5B shows that in the Mu-p53-2 clone no induction of DNA fragmentation was present in both 5-FU (at IC50 concentrations) and in the combination-treated cells. Further support to indicate apoptotic induction was provided by the characteristic feature, revealing typical apoptotic changes, observed under phase contrast microscopy.

### Table 1

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<tr>
<th>Cell Line</th>
<th>2 Days IC50</th>
<th>6 Days IC50</th>
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<tbody>
<tr>
<td>HCT-116/Par</td>
<td>19.9 ± 1.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>/CMV-2</td>
<td>17.7 ± 2.3</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>/CMV-4</td>
<td>16.7 ± 2.5</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>/Mu-p53-2</td>
<td>3.0 ± 0.8</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>/Mu-p53-4</td>
<td>222 Russo et al.</td>
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in cell nuclei stained with 4,6-diamidino-2-phenylindole (data not shown).

**Discussion**

Results show that the farnesyltransferase inhibitor RPR-115135 enhances the antiproliferative effects of 5-FU in the human colon cancer HCT-116 isogenic cell system. The effect, obtained at equitoxic drug concentrations, was more pronounced in p53 wild-type cells.

The isogenic cell line system HCT-116, in which p53 function was disrupted by transfection with a dominant-negative mutant p53 transgene, harbors a K-Ras mutation. The isogenic cell clones with p53 alterations were found to be more

### Table 2

<table>
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<tr>
<th>Drug</th>
<th>CMV-2</th>
<th>Mu-p53-2</th>
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<tr>
<td>5-FU IC_{12.5}</td>
<td>85.2 ± 1.5</td>
<td>91.9 ± 0.5</td>
</tr>
<tr>
<td>5-FU IC_{25}</td>
<td>29.5 ± 2.3</td>
<td>40.0 ± 3.6</td>
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<tr>
<td>5-FU IC_{50}</td>
<td>19.4 ± 0.5</td>
<td>19.8 ± 4.8</td>
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<tr>
<td>RPR IC_{12.5}</td>
<td>94.8 ± 2.5</td>
<td>92.5 ± 4.6</td>
</tr>
<tr>
<td>RPR IC_{25}</td>
<td>88.3 ± 5.2</td>
<td>87.6 ± 5.4</td>
</tr>
<tr>
<td>RPR IC_{50}</td>
<td>60.8 ± 6.4</td>
<td>73.8 ± 4.3</td>
</tr>
<tr>
<td>5-FU IC_{12.5} + RPR IC_{12.5}</td>
<td>16.7 ± 1.1</td>
<td>57.1 ± 3.3</td>
</tr>
<tr>
<td>5-FU IC_{25} + RPR IC_{25}</td>
<td>8.0 ± 1.4</td>
<td>12.4 ± 2.5</td>
</tr>
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</table>

RPR, RPR-115135.
Fig. 3. Analysis of cell cycle perturbation induced by RPR-115135, 5-FU, or 5-FU + RPR-115135 (equitoxic concentrations) after 6 days continuous exposure. Each point represents the mean ± S.E. of at least three independent experiments, performed in duplicate. A, CMV-2 clone: 1) CTRL, 2) 5-FU IC_{12.5}, 3) RPR-115135 IC_{12.5}, 4) 5-FU IC_{12.5} + RPR-115135 IC_{12.5}, and 5) 5-FU IC_{50}. B, Mu-p53-2 clone: 1) CTRL, 2) 5-FU IC_{25}, 3) RPR-115135 IC_{25}, 4) 5-FU IC_{25} + RPR-115135 IC_{25}, and 5) 5-FU IC_{50}. Each point represents the mean ± S.E. of at least three independent experiments performed in duplicate.

Fig. 4. Induction of sub-G₁ cell population induced by RPR-115135, 5-FU, or 5-FU + RPR-115135 (equitoxic concentrations) over 6 days of exposure, evaluated by cell flow cytometry (A, CMV-2; B, Mu-p53-2 cells). Each point represents the mean ± S.E. of at least three independent experiments performed in duplicate.
resistant to 5-FU compared with parental or control-transfected cells only when cells are treated with low concentration of 5-FU over a long period (>4 days). This finding is in agreement with previous studies, indicating that cell death in intestinal epithelia requires the incorporation of 5-FU metabolites into RNA (Pritchard et al., 1997, 1998). For RNA incorporation of 5-FU metabolites to occur, exposure of cells to 5-FU must be prolonged (4–7 days) (Ren et al., 1997). Resulting cell death is by apoptosis and is p53-dependent. In the CMV-2 clone 5-FU induces a strong G2-M arrest (p53-dependent) and a moderate apoptosis, whereas in the p53-mutated clone 5-FU induces an S increase (p53-independent) and no apoptosis. However, the difference between the two clones is not as substantial as these data suggest.

It was previously shown that HCT-116 cells are not prone to apoptosis induced by γ-irradiation (Fan et al., 1997, 1998), probably due to the fact that HCT116 cells have lost the normal functions of the gene products of p14ARF and p16INK4 (Myohanen et al., 1998; Yang et al., 2000). The INK4A/ARF locus, on human chromosome 9p21, consists of two overlapping genes that encode two unrelated proteins p16INK4A and p14ARF (the mouse homolog p19). Mice heterozygous for ARF developed lymphomas as rapidly as p53 mice, suggesting that loss of p14ARF expression might have similar consequences of loss of p53 (Schmitt et al., 1999; Khan et al., 2000; Lloyd, 2000; Ries et al., 2000). ARF promotes MDM2 degradation and thus prevents the MDM2-mediated neutralization of p53 (Schmitt et al., 1999; Khan et al., 2000; Lloyd, 2000; Ries et al., 2000). Alterations in ARF function would result in overexpression of MDM2 and functional inactivation of p53. It has been observed that induction of p53/p21<sup>kip1/waf1</sup>-dependent G2-M arrest by γ-irradiation does not depend on ARF (Khan et al., 2000; Lloyd, 2000) indicating that, at least in the case of DNA damage induced by γ-irradiation, ARF plays at most a redundant, if not a minor role in mediating p53 accumulation. The p53 pathway plays a role in inducing DNA damage in HCT-116 cells, albeit a minor one compared with that demonstrated in MCF-7 cells (Fan et al., 1997, 1998).

Taken together, the above-mentioned considerations indicate that loss of ARF in tumor cells containing wild-type p53 causes a deregulation of MDM2, thus inhibiting p53 from exerting completely its effects after DNA damage. Very recently, Ries et al. (2000), working in HCT-116 cells, identified the loss of p14ARF as a mechanism that allows dl1520 [adenovirus mutant dl1520 (ONYX-015), this virus replicates in tumor cells with mutant p53 but not in normal cells with functional p53] replication in tumor cells retaining wild-type p53.

That RPR-115135, a nonpeptidomimetic inhibitor of FTase, acts synergistically with 5-FU to induce apoptosis of CMV-2 clone, appears to be a particularly important finding. Although RPR-115135 itself does not induce significant levels of apoptosis (or cell growth inhibition and cell cycle alterations) at the concentrations used (IC<sub>12.5</sub> or IC<sub>25</sub>) it enhances 5-FU-induced apoptosis. This effect was obtained with very low concentrations of RPR-115135 and 5-FU (0.18 and 0.3 μM, respectively). The effects induced by the combination

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**Fig. 5.** Induction of DNA secondary fragmentation (apoptosis-related), evaluated by filter binding assay induced by RPR-115135, 5-FU, or 5-FU + RPR-115135 (equitoxic concentrations) over 6 days of exposure (A, CMV-2; B, Mu-p53-2 cells). Each point represents the mean ± S.E. of at least three independent experiments performed in duplicate.
were stronger than those induced by 5-FU alone at the IC_{50} (1.2 μM). In the Mu-p53-2 clone RPR-11535 in association with 5-FU was unable to induce apoptosis. In addition, higher concentrations of RPR-11535 (IC_{25} of 0.2 μM) and higher concentrations of 5-FU (IC_{25} of 1.5 μM), with respect to those used in the CMV-2 clone, were less active than 5-FU alone at the IC_{50} (2.6 μM) concentration.

Although RPR-11535 can potentiate the effect of 5-FU in a clone in which p53 function is disrupted, our data strongly suggest that RPR-11535 significantly enhances the efficacy of 5-FU only when p53 is functioning. Overall, results indicate that even when p53 is not perfectly functioning in HCT-116 cells, RPR-11535 enhances 5-FU-induced apoptosis.

These results appear to have important clinical implications because the use of FTase inhibitors in association with cytotoxic agents could enhance the antineoplastic properties of these drugs. It has been demonstrated that several cytotoxic agents (taxol, cisplatin, and gemcitabine) show increased antitumor activity when used in association with FTase inhibitors, both in vitro and in mouse tumor xenograft models (Peeters et al., 1999). Results from the first clinical studies of FTase inhibitors used in association with known cytotoxics have been the object of debate at the 1999 annual meeting of the American Association of Clinical Oncology (1999). A phase I trial of an FTase inhibitor in combination with 5-FU/leucovorin carried out in advanced colorectal cancer demonstrated that hematotoxicity was the dose-limiting toxicity (Peeters et al., 1999).

Our preclinical results could have important clinical implications. The status of p53 in cancer cells appears to significantly affect drug action. RRP-11535, although its activity is p53-independent, was very effective in potentiating 5-FU in the wild-type CMV-2 clone. The schedule of administration of 5-FU with RPP-11535 appears important. A significant increase in 5-FU activity is observed only when 5-FU is acting in a p53-dependent manner (RNA incorporation, long incubation time). It appears that a clinical trial of continuous infusion 5-FU in association with an FTase inhibitor in colorectal tumors that maintain p53 function is warranted.

References


