The Farnesyltransferase Inhibitor R115777 Up-regulates the Expression of Death Receptor 5 and Enhances TRAIL-Induced Apoptosis in Human Lung Cancer Cells

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Abstract

Tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) preferentially induces apoptosis in transformed or malignant cells, thus exhibiting potential as a tumor-selective apoptosis-inducing cytokine for cancer treatment. Many studies have shown that the apoptosis-inducing activity of TRAIL can be enhanced by various cancer therapeutic agents. R115777 (tipifarnib) is the first farnesyltransferase inhibitor (FTI) that showed clinical activity in myeloid malignancies. In general, R115777, like other FTIs, exerts relatively weak effects on the induction of apoptosis in cancer cells with undefined mechanism(s). In the current study, we studied its effects on the growth of human lung cancer cells, including induction of apoptosis, and examined potential underlying mechanisms for these effects. We showed that R115777 induced apoptosis in human lung cancer cells, in addition to inducing G1 or G2-M arrest. Moreover, we found that R115777 up-regulated the expression of death receptor 5 (DR5), an important death receptor for TRAIL, and exhibited an augmented effect on the induction of apoptosis when combined with recombinant TRAIL. Blockage of DR5 induction by small interfering RNA (siRNA) abrogated the ability of R115777 to enhance TRAIL-induced apoptosis, indicating that R115777 augments TRAIL-induced apoptosis through up-regulation of DR5 expression. Thus, our findings show the efficiency of R115777 in human lung cancer cells and suggest that R115777 may be used clinically in combination with TRAIL for treatment of human lung cancer. [Cancer Res 2007;67(10):4973–80]

Introduction

The tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) receptor death receptor 5 (DR5, also named TRAIL-R2, TRICK2, or Killer/DR5) is one of the death receptors that share a similar, cysteine-rich extracellular domain and additional cytoplasmic death domain (1). DR5 locates at the cell surface, becomes activated or oligomerized (trimerized) upon binding to its ligand TRAIL or overexpression, and then signals apoptosis through caspase-8–mediated rapid activation of caspase cascades (1, 2).

Recently, DR5 has attracted much more attention because its ligand TRAIL preferentially induces apoptosis in transformed or malignant cells, thus demonstrating potential as a tumor-selective apoptosis-inducing cytokine for cancer treatment (3, 4). Certain cancer therapeutic agents induce the expression of DR5 in cancer cells and are thereby able to augment TRAIL-induced apoptosis or initiate apoptosis (5–7).

Farnesyltransferase inhibitors (FTI) are a class of agents that suppress the farnesyltransferase enzyme to prevent certain proteins such as the Ras oncoprotein from undergoing farnesylation (8–10). These agents inhibit proliferation and induce apoptosis in various types of cancer cell lines in culture or suppress the growth of xenografts in nude mice with limited toxicity (8–10). In the clinic, FTIs are well tolerated and have some positive outcomes in certain settings, such as hematologic malignancies and breast cancer, although the response rates to FTIs alone are generally poor. When combined with other therapeutic agents or radiotherapy, FTIs exhibit some encouraging clinical responses (8, 10). Although FTIs were historically developed as anti-Ras agents, it is now generally agreed upon that FTIs exert their antitumor activity independent of their activity on inhibiting Ras farnesylation (8, 9). Otherwise, the mechanisms underlying the antitumor effects of FTIs remain largely undefined.

R115777 is one of the clinically tested FTIs and is the first one to show clinical activity in myeloid malignancies (11). Preclinical studies have shown that this agent inhibited the growth of the majority of tested human cancer cell lines (12), induced apoptosis in certain types of cancer cells (13–15), and suppressed the growth of tumor xenografts in nude mice with an increase in apoptotic index (12, 16). When R115777 was combined with other therapeutic agents, such as taxol and the proteasome inhibitor bortezomib, enhanced effects on growth inhibition or apoptosis induction were observed (17–19). Moreover, R115777 was also effective in inhibiting the growth of chemical-induced lung carcinogenesis in mice (20), suggesting potential activity as a chemopreventive agent. In the clinic, the most promising activity of R115777 has been seen primarily in hematologic malignancies (8, 11). Although R115777 as a single agent exhibited limited clinical activity in solid tumors, when used in combination with other agents such as tamoxifen, it did induce responses in some patients with solid tumors (8).

In an effort to elucidate the molecular mechanisms of FTI-induced growth arrest and apoptosis and to develop mechanism-oriented, FTI-based combination regimens for effective treatment of cancer, we found that R115777 up-regulated DR5 expression, including causing an increase in cell surface DR5 levels. Accordingly, R115777 cooperated with TRAIL to enhance induction of apoptosis in human lung cancer cells. Thus, our findings in this
study provide a scientific rationale for the combination of R115777 and TRAIL for the treatment of human lung cancer and possibly other types of cancer as well.

Materials and Methods

Reagents. R115777 was provided by Johnson & Johnson Pharmaceutical Research and Development, LLC. It was dissolved in DMSO at a concentration of 10 mmol/L, and aliquots were stored at −80°C. Stock solutions were diluted to the desired final concentrations with growth medium just before use. Soluble recombinant human TRAIL was purchased from PeproTech Inc. Rabbit polyclonal anti-DR5 antibody was purchased from ProSci Inc. Mouse monoclonal anti–caspase-3 was purchased from ProSci Inc. Rabbit polyclonal anti–caspase-9, anti–caspase-8, and anti–poly(ADP-ribose) polymerase (PARP) antibodies were purchased from Cell Signaling Technology, Inc.. Mouse monoclonal anti–RasGAP antibody (B4F8) was purchased from Santa Cruz Biotechnology. Mouse monoclonal anti–HDJ-2 antibody (clone KA2A5.6) was purchased from Lab Vision Corp. Mouse monoclonal anti–FLICE inhibitory protein (FLIP) antibody (NF6) was purchased from Alexis Biochemicals. Rabbit polyclonal anti–β-actin antibody was purchased from Sigma Chemical Co.

Cell lines and cell cultures. All human lung cancer cell lines used in this study were purchased from the American Type Culture Collection. H157-Lac Z-5 and H157-FLIPL-6, which stably express Lac Z and FLIP L, respectively, were described previously (21). These cell lines were grown in monolayer culture in RPMI 1640 supplemented with glutamine and 5% fetal bovine serum (FBS) at 37°C in a humidified atmosphere consisting of 5% CO2 and 95% air.

Western blot analysis. The procedures for preparation of whole-cell protein lysates and Western blot analysis were described previously (22, 23).

Detection of DR5 mRNA expression. DR5 mRNA was detected using reverse transcription-PCR (RT-PCR) described as follows. Total RNA was isolated from cells using TRI Reagent (Sigma Chemical Co.) as instructed by the manufacturer. First-strand cDNA was synthesized from 2 μg of total RNA using iScript cDNA Synthesis Kit (Bio-Rad Laboratories) following the manufacturer’s instructions. The given cDNAs were then amplified by PCR.
using the following primers: DR5 sense 5'-GACCTAGCTCCCCAGCAGA- 
GAG-3', DR5 antisense 5'-CGGCTGCAACTGCTGACTTAT-3', β-actin 
sense, 5'-GAACATCTTAACTCCATC-3', and β-actin antisense 5'-CTA- 
GACATTTGGGCTGGACATGGAGGGCC-3'. The 25-μL amplification 
mixture contained 2 μL of cDNA, 0.5 μL of deoxynucleotide triphosphate 
(25 mmol/L each), 1 μL each of the sense and antisense primers (20 μmol/L 
each), 5 μL of TaqMaster PCR enhancer, 1 μL of Taq DNA polymerase 
(5 units/μL; Eppendorf), 2.5 μL 10× reaction buffer, and sterile H2O. PCR 
did not last for 28 cycles. After an initial step at 95°C for 3 min, each cycle 
consisted of 50 s of denaturation at 94°C, 50 s of annealing at 58°C, and 55 s 
of extension at 72°C. This was followed by an additional extension step at 
72°C for 10 min. The housekeeping gene β-actin was also amplified as an 
internal reference. PCR products were resolved by electrophoresis on a 2% 
agarose gel, stained, and directly visualized under UV illumination.

Construction of DR5 reporter plasmids, transient transfection, and 
luciferase activity assay. The plasmid containing a 5'-flanking region of 
DR5 gene was kindly provided by Dr. G.S. Wu (Wayne State University 
School of Medicine, Detroit, MI). We then used this plasmid as a template to 
amplicate different lengths of the 5'-flanking region of the DR5 gene by 
PCR and then subcloned these fragments, respectively, into pGL3-basic reporter 
vector (Promega) through XhoI and BglII restriction sites. In the PCR 
amplification, the reverse primer 5’-CTTAAGATCTGGCGGTAGG- 
GAACGCTCTTATAGTC-3’ was used to make all deletion constructs. The 
upstream primers used were 5’-CTTAGAATCTCCTGCCGGAAG- 
ACAGCCCGGG-3’ (−3,070), 5’-CTTAGAATCTCCTGCCGGAAG- 
ACAGCCCGGG-3’ (−420), and 5’-CTTAGAATCTCCTGCCGGAAG- 
ACAGCCCGGG-3’ (−373), respectively. These constructs were then named 
pGL3-DR5(−3,070), pGL3-DR5(−420), and pGL3-DR5(−373), respectively.

For examining the effect of R115777 on DR5 transactivation activity, cells 
were seeded in 24-well plates and cotransfected with the given reporter 
plasmid (0.5 μg per well) and pCH110 plasmid encoding β-galactosidase 
(β-gal; Pharmacia Biotech; 0.2 μg per well) using FuGene 6 transfection 
reagent (3:1 ratio; Roche Molecular Biochemicals) following the manufac-
turer's protocol. Twenty-four hours later, the cells were treated with 
R115777. After 12 h, the cells were lysed and subjected to luciferase activity 
assay using Luciferase Assay System (Promega) in a luminometer. Relative 
luciferase activity was normalized to β-gal activity, which was measured as 
described previously (24).

Detection of cell surface DR5. Cell surface DR5 expression was 
analyzed using flow cytometry as described previously (25). The mean 
fluorescence intensity (MFI) that represents antigenic density on a per-cell 
basis was used to represent DR5 expression level. Phycoerythrin-conjugated 
mouse anti-human DR5 monoclonal antibody (DJR2-4), anti-human DR4 
monoclonal antibody (DJR1), and mouse immunoglobulin G1 (IgG1) isotype 
control (MOPC-21/P3) were purchased from eBioscience.

Detection of caspase activation and apoptosis. Caspase activation 
and their substrate cleavage were detected by Western blot analysis as 
described above. Apoptosis was detected using an annexin V- 
phycocerythrin Apoptosis Detection kit purchased from BD Biosciences following 
the manufacturer's instructions. In addition, sub-G1 was also measured by 
flow cytometry as described previously (26) as another indication of 
apoptosis.

Cell survival assay. Cells were seeded in 96-well cell culture plates and 
treated on the second day with the indicated agents. At the end of 
treatment, cell number was estimated by the sulforhodamine B assay as 
previously described (27). The cell survival was presented as the percentage

![Image](https://www.aacrjournals.org/FIG2.png)

**Figure 2.** R115777 increases DR5 expression at both protein (A and B) and mRNA (C) levels and transactivates DR5 promoter (D) in human lung cancer cells. A and B: the indicated cell lines were treated with the given concentrations of R115777 for 16 h (A), or H1792 cells were treated with 5 μmol/L R115777 for the indicated times (B). Whole-cell protein lysates were then prepared from aforementioned treatments for detection of DR5 and HDJ-2 using Western blot analysis. U, unprocessed; P, processed. C: the indicated cell lines were exposed to the given concentrations of R115777 for 12 h. Cellular total RNA was then prepared for detection of DR5 mRNA using RT-PCR. Actin levels were used as an internal control. D, the given reporter constructs with different lengths of the 5'-flanking region of the DR5 gene were cotransfected with pCH110 plasmid into H1792 cells. After 24 h, the cells were treated with DMSO or 10 μmol/L R115777 for 12 h and then subjected to luciferase assay. Columns, means of triplicate determinations; bars, SD.
R115777 effectively inhibits the growth of human lung cancer cells through induction of apoptosis and growth arrest. The effects of R115777 on the growth of human lung cancer cells have not been systemically evaluated. Therefore, we examined the effects of R115777 on the growth of a panel of human non–small cell lung cancer cells by different assays. R115777 effectively inhibited the growth of six tested cell lines by measuring cell number, with IC_{50} ranging from 2 to 6 μmol/L after a 3-day exposure (Fig. 1A). Under normal culture condition (i.e., 5% FBS), R115777 in general was a weak inducer of apoptosis because it induced apoptosis in some cell lines (e.g., H358 and H1299) but not in others (e.g., A549 and H157). However, under low-serum (i.e., 0.1% culture conditions, the effects of R115777 on apoptosis induction were substantially enhanced in all of the tested cell lines. Under both culture conditions, A549 and H157 were relatively resistant to R115777-induced apoptosis (Fig. 1B). In addition to induction of apoptosis, R115777 induced cell cycle arrest either at the G1 phase (i.e., H157, A549, and H1299) or at the G2-M phase (i.e., H460, H1792, and H358), indicating that R115777 induces growth arrest. Collectively, these results show that R115777 inhibits the growth of human lung cancer cells through the induction of apoptosis and/or growth arrest.

**R115777 induces DR5 expression in human lung cancer cells.** To understand the mechanism by which R115777 induces apoptosis, we screened its effects on the expression of certain genes related to apoptosis. Our preliminary results indicate that DR5 is a gene up-regulated in cells exposed to R115777. Therefore, we did detailed experiments to study the effects of R115777 on the expression of DR5 in a panel of human lung cancer cells. We found that R115777 at concentrations ranging from 2.5 to 10 μmol/L increased DR5 protein levels in a concentration-dependent manner. The most dramatic increase in DR5 levels post–R115777 exposure were observed in H1792 cells and H1299 cell lines (Fig. 2A). R115777 induced a weak increase in DR5 levels in H157 cells. Time course analysis indicated that R115777 started to increase DR5 levels at 3 h, which was sustained up to 24 h (Fig. 2B). Under these conditions, the farnesylation of HDJ-2 protein was apparently inhibited (Fig. 2A and B), indicating that R115777 indeed inhibits protein farnesylation in the tested cell lines. Moreover, we determined whether R115777 up-regulated DR5 at the transcriptional level. R115777 increased not only DR5 mRNA levels evaluated by RT-PCR (Fig. 2C), but also the luciferase activity of the cells transfected with reporter plasmids with different lengths of the 5′-flanking region of the DR5 gene ranging from 3,070 to 375 bp upstream of the translation start site (Fig. 2D). These results show that R115777 induces DR5 expression at the transcriptional level.

**R115777 induces cell surface DR5 distribution.**Because DR5 is a functional protein on the cell surface, we then analyzed cell surface DR5 levels in cells exposed to R115777. As shown in Fig. 3A, both H1792 and H157 cells treated with R115777 exhibited increased fluorescent intensity of DR5 staining in comparison with DMSO-treated cells (i.e., DR5 staining peak shifted to the right). The MFIs in both H1792 and H157 cells were increased close to 4-fold over those in DMSO-treated cells (Fig. 3B). These results clearly indicate that R115777 increases the amounts of DR5 on the cell surface. We also analyzed the effects of R115777 on the distribution of cell surface DR4, a protein with similar functions to DR5, in these cell lines. R115777 increased cell surface DR4 in H157 cells (1.95-fold), but only minimally in H1792 cells (1.32-fold; Fig. 3). Thus, these results indicate that R115777 primarily increases cell surface DR5 in human lung cancer cells.

**R115777 cooperates with TRAIL to induce apoptosis in human lung cancer cells.** Because R115777 increases cell surface DR5, we hypothesized that R115777 would sensitize cells to TRAIL-induced apoptosis. Therefore, we examined the effects of the
combination of R115777 and TRAIL on cell survival and apoptosis in human lung cancer cells. As presented in Fig. 4A, the combination of R115777 and TRAIL worked better than each single agent in decreasing cell survival. Accordingly, the combination was more potent than each single agent in inducing apoptosis estimated by annexin V staining (Fig. 4B). For example, R115777 at 5 μmol/L and TRAIL at 20 ng/mL alone induced 11.5% and 22.4% of cells to undergo apoptosis, respectively, whereas their combination induced 40% of cells to undergo apoptosis. Thus, it seems that the combination of R115777 and TRAIL synergistically induces apoptosis. Moreover, we examined the effects of R115777 and TRAIL combination on the activation of caspase cascades. R115777 alone at concentrations ranging from 2.5 to 10 μmol/L did not cause cleavage of the tested caspases. TRAIL alone at 20 ng/mL weakly induced cleavage of caspases and caspase-3 substrates, PARP, and RasGAP. However, their combinations exhibited enhanced effects on cleavage of these proteins (Fig. 4C). As the concentrations of R115777 in the combinations were increased, the cleavage of the caspases and related proteins were more pronounced. Thus, the combination of R115777 and TRAIL augments activation of caspases, further indicating that R115777 cooperates with TRAIL to induce apoptosis.

R115777 enhances TRAIL-induced apoptosis through up-regulation of DR5. To determine whether R115777 enhances TRAIL-induced apoptosis through DR5 up-regulation, we examined the effects of R115777 and TRAIL combination on apoptosis induction in cells where DR5 expression was silenced with DR5 siRNA. In control siRNA-transfected cells, R115777 increased DR5 levels (Fig. 5A, lane 2). In DR5 siRNA-transfected cells, the basal levels of DR5 were reduced (Fig. 5A, lane 5) and not increased further by R115777 (Fig. 5A, lane 6). These results indicate a successful silencing of DR5 expression. By apoptotic assay, we detected up to 70% apoptotic cells in control siRNA-transfected cells, but only 28% apoptotic cells in DR5 siRNA-transfected cells.

Figure 4. Combination of R115777 and TRAIL exerts augmented effects on decreasing cell survival (A), inducing apoptosis (B), and activating caspases (C). A, the indicated cell lines were treated with the indicated concentrations of R115777 alone, 25 ng/mL TRAIL alone, and their respective combinations as indicated. After 24 h, cell number was estimated using sulforhodamine B assay for calculation of cell survival; B, H157 cells were treated with the indicated concentrations of R115777 alone, 20 ng/mL TRAIL alone, and their respective combinations as indicated. After 24 h, the cells were harvested for measurement of apoptosis using annexin V staining. The percent positive cells in the top right and bottom right quadrants were added to yield the total of apoptotic cells. The cells in the bottom left quadrant were surviving cells; C, H157 cells were treated with the indicated concentrations of R115777 alone, 20 ng/mL TRAIL alone, and their respective combinations for 16 h. The cells were then subjected to preparation of whole-cell protein lysates and subsequent Western blot analysis for detecting cleavage of caspases and their substrates.

Casp, caspase.
upon treatment with R115777 and TRAIL combination (Fig. 5B).
Collectively, these results clearly indicate that up-regulation of DR5 is
a key event that mediates augmentation of apoptosis induced by
the combination of R115777 and TRAIL. We noted that more
apoptotic cells were detected in control siRNA-transfected H157
cells treated with either TRAIL or the combination of R115777 and
TRAIL compared with the result presented in Fig. 4B generated
from the same cell line exposed to the similar treatment. In
another transfection experiments, we generated identical results
from control siRNA-transfected H157 cells treated with the same
combination. Therefore, it is possible that the transfected cells are
somewhere more susceptible than their parental cells to undergo
apoptosis upon treatment with TRAIL or the combination of
R115777 and TRAIL. Of course, the discrepancy may also be caused
by varied activities of different batches of recombinant TRAIL.

R115777 modulates c-FLIP expression in a cell line–dependent manner. c-FLIP including FLIPL and FLIPS are key
proteins that negatively regulate the extrinsic death receptor–
mediated apoptotic pathway by inhibiting caspase-8 activation
(29). Some cancer therapeutic agents enhance cell sensitivity to
TRAIL-induced apoptosis via down-regulation of c-FLIP expression
(30–33). Therefore, we further examined the effects of R115777 on
c-FLIP expression in human lung cancer cells. In H1792 cells,
R115777 at the given concentrations decreased the levels of both
FLIPL and FLIPS. However, R115777 slightly increased the levels of
both forms of c-FLIP in H157 and A549 cells. We detected neither
basal levels of c-FLIP nor clear modulation by R115777 in H1299
cells (Fig. 6A). Thus, it seems that R115777 exerts a cell line–
dependent modulation of c-FLIP in human lung cancer cells.

Enforced expression of exogenous c-FLIP protects cells
from apoptosis induced by the combination of R115777 and
TRAIL. Because the combination of R115777 and TRAIL still
augmented apoptosis in the H157 cell line, in which c-FLIP levels
were increased, we examined whether enforced expression of
exogenous c-FLIP inhibited apoptosis induced by the combination
of R115777 and TRAIL. By means of lentiviral infection, we
established a stable H157 cell line that expressed high levels of
exogenous FLIPL (Fig. 6B). In Lac Z (control)–transfected cell line,
we detected ~2%, 10%, 14%, and 42% annexin V–positive
(apoptotic) cells from cells treated with DMSO, R115777, TRAIL,
and the combination of R115777 and TRAIL, respectively. However,
annexin V–positive cells were ~2%, 3%, 2%, and 6% in FLIPL–
transfected H157 cells treated with DMSO, R115777, TRAIL, and
the combination of R115777 and TRAIL, respectively (Fig. 6C).
These results clearly indicate that enforced expression of FLIPL
abolished apoptosis induced by R115777 and TRAIL.

Discussion

The effects of R115777 on the growth including apoptosis and
cell cycle distribution of human lung cancer cells have not been
fully evaluated in a preclinical setting. In this study, we show that
R115777, at a clinically achievable and safe concentration range
(2–6 μmol/L; refs. 34–37), effectively inhibited the growth of
human lung cancer cells, primarily through inducing growth arrest
and apoptosis. In general, R115777 was not a potent inducer of
apoptosis under normal serum culture condition, although its
apoptosis-inducing effects could be substantially enhanced by low-
sodium culture condition. Moreover, R115777 induced cell cycle
arrest either at the G1 phase or at the G2-M phase depending on cell
lines. All of these phenomena are consistent with those observed
from other FTIs (26, 38, 39).

In this study, we show, for the first time, that R115777 induces
DR5 expression not only at protein levels but also at mRNA levels.
Moreover, R115777 increased transactivation of the DR5 promoter,
indicating that R115777 modulates DR5 expression at the
transcriptional level. In addition, we showed that R115777
increased amounts of DR5 at the cell surface, indicating that
R115777 induces cell surface DR5 distribution. We noted that
R115777 exerted only a moderate effect on modulation of DR5
protein levels in H157 cells (Fig. 1A). However, it increased cell
surface DR5 in H157 cells as strongly as in H1792 cells (Fig. 3).
These results suggest that R115777 induces DR5 redistribution at
the cell surface in addition to increasing DR5 expression.

![Figure 5](cancerres.aacrjournals.org) Silencing of DR5 expression by siRNA (A) attenuates apoptosis induced by the combination of R115777 and TRAIL (B). H157 cells were seeded in a 24-well
plate culture and on the second day transfected with control (Ctrl) or DR5 siRNA. Forty hours later, the cells were treated with 10 μmol/L R115777, 20 ng/mL
TRAIL, and their combination. After 15 h, the cells were harvested for preparation of whole-cell protein lysates and subsequent Western blot analysis (A) or for detection
of apoptotic cells using annexin V staining (B). In annexin V assay, the percent positive cells in the top right and bottom right quadrants were added to yield the
total of apoptotic cells. The cells in the bottom left quadrant were surviving cells.
In addition to R115777, we found that another FTI called SCH66336 (lonafarnib) also induced DR5 expression and cell surface DR5 distribution. Both agents have farnesyltransferase-inhibitory activity; however, they have distinct chemical structures. In our study, R115777 rapidly increased DR5 expression at 3 h posttreatment, which was accompanied by inhibition of HDJ-2 protein farnesylation (Fig. 3B). Therefore, we suggest that DR5 up-regulation by R115777 is associated with its ability to inhibit protein farnesylation. Because R115777 functions like SCH66336 to increase DR5 mRNA as well, it is unlikely that R115777 directly modulates DR5 protein. Rather, it may inhibit the farnesylation of an unknown protein, leading to increased DR5 transcription. Nevertheless, our findings on R115777 as well as SCH66336 warrant further study on the relationship between protein farnesylation and DR5 modulation.

Preliminary results from clinical trials have shown that R115777 exhibits promising efficacy in hematologic malignancies with a favorable toxicity profile; however, they have distinct chemical structures. In our study, R115777 rapidly increased DR5 expression at 3 h posttreatment, which was accompanied by inhibition of HDJ-2 protein farnesylation (Fig. 3B). Therefore, we suggest that DR5 up-regulation by R115777 is associated with its ability to inhibit protein farnesylation. Because R115777 functions like SCH66336 to increase DR5 mRNA as well, it is unlikely that R115777 directly modulates DR5 protein. Rather, it may inhibit the farnesylation of an unknown protein, leading to increased DR5 transcription. Nevertheless, our findings on R115777 as well as SCH66336 warrant further study on the relationship between protein farnesylation and DR5 modulation.

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Preliminary results from clinical trials have shown that R115777 exhibits promising efficacy in hematologic malignancies with a favorable toxicity profile (8, 11). R115777, at a clinically achievable and safe concentration range (2-6 μmol/L; refs. 34–37), up-regulated DR5 expression and induced cell surface DR5 distribution. Accordingly, we showed that the combination of a clinically achievable concentration of R115777 with TRAIL exhibited augmented effects on decreasing cell survival and inducing apoptosis (Fig. 4). Given that TRAIL is considered as a cancer-selective cytokine with cancer therapeutic potential and is being tested in phase I clinical trials, our current finding that R115777 enhances TRAIL-induced apoptosis clearly has translational significance in the clinic.

Some studies have shown that certain cancer therapeutic agents enhance TRAIL-induced apoptosis via down-regulation of c-FLIP expression (30–32, 40). In our study, we found that R115777 induced DR5 expression and cell surface distribution in all of the tested cell lines. However, it modulated c-FLIP expression in a cell line-dependent manner. R115777 even slightly increased c-FLIP levels in some cell lines (e.g., H157), whereas it decreased c-FLIP expression in other cell lines (e.g., H1792). Regardless of the differential modulation of c-FLIP expression, the combination of R115777 and TRAIL exerted augmented effects on decreasing cell survival and inducing apoptosis in these lung cancer cell lines, suggesting that it is unlikely for R115777 to enhance TRAIL-induced apoptosis via modulation of c-FLIP levels in certain cell lines in which c-FLIP levels are not decreased. Through

Figure 6. Differential effects of R115777 on c-FLIP levels (A) and protection of enforced c-FLIP expression (B) on apoptosis induced by the combination of R115777 and TRAIL (C). A, the indicated cell lines were treated with the given concentrations of R115777. After 16 h, the cells were subjected to preparation of whole-cell protein lysates and subsequent Western blot analysis. B, whole-cell protein lysates were prepared from H157 cells transfected with Lac Z and FLIPL, respectively, and then subjected to detection of FLIPL expression by Western blot analysis. C, H157-Lac Z-5 or H157-FLIPL-6 cells were treated with DMSO, 10 μmol/L R115777, 5 ng/mL TRAIL, and the combination of R115777 and TRAIL, respectively. After 24 h, the cells were harvested and subjected to detection of apoptotic cells using annexin V staining. The percent positive cells in the top right and bottom right quadrants were added to yield the total of apoptotic cells. The cells in the bottom left quadrant were surviving cells.

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silencing DR5 expression and blocking DR5 induction by R115777 using siRNA targeting DR5, our data clearly show that apoptosis induced by the combination of R115777 and TRAIL was substantially attenuated. This result thus indicates that R115777 enhances TRAIL-induced apoptosis primarily via up-regulation of DR5.

Our results show that the combination of R115777 and TRAIL augmented induction of apoptosis in H157 cells, in which c-FLIP levels were actually increased upon R115777 treatment. When FLIPL expression was enforced to high levels in this cell line, either TRAIL alone or the combination of R115777 and TRAIL failed to induce apoptosis, indicating that FLIPL overexpression indeed inhibits TRAIL/death receptor-mediated apoptosis. Therefore, we suggest that R115777-induced DR5 expression and cell surface redistribution override c-FLIP up-regulation, leading to enhancement of TRAIL-induced apoptosis in cell lines where c-FLIP expression is high or increased by R115777. If this is correct, we assume that cell lines in which c-FLIP levels are reduced upon R115777 treatment (e.g., H1792) will be more susceptible than other cell lines where c-FLIP expression is increased or not altered by R115777 (e.g., H157) to apoptosis induction by the combination of R115777 and TRAIL as shown in Fig. 4A.

Among the tested lung cancer cell lines, A549 and H157 cell lines are the least sensitive to R115777-induced apoptosis even under low-serum culture condition (Fig. 1C). We noted that these two cell lines had relatively higher levels of c-FLIP compared with H1792 and H1299 cells (Fig. 6A), which are sensitive to R115777-induced apoptosis (Fig. 1C). Whether these results suggest that the levels of c-FLIP determine cell sensitivity to R115777-induced apoptosis needs to be investigated in the future.

In summary, we have shown that R115777 increases DR5 expression, induces DR5 distribution at the cell surface, and subsequently enhances TRAIL-induced apoptosis. These findings warrant clinical evaluation of the efficacy of R115777 and TRAIL in the treatment of human lung and other types of cancer in the future.

Acknowledgments

Received 11/1/2006; revised 1/19/2007; accepted 3/2/2007.

Grant support: Georgia Cancer Coalition Distinguished Cancer Scholar award (S-Y. Sun) and Department of Defense grant W81XWH-04-1-0142-VITAL (S-Y. Sun for Project 4).

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The Farnesyltransferase Inhibitor R115777 Up-regulates the Expression of Death Receptor 5 and Enhances TRAIL-Induced Apoptosis in Human Lung Cancer Cells

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