Induction of Fas Expression and Augmentation of Fas/Fas Ligand-mediated Apoptosis by the Synthetic Retinoid CD437 in Human Lung Cancer Cells

Shi-Yong Sun,2 Ping Yue, Waun Ki Hong, and Reuben Lotan

Department of Thoracic/Head and Neck Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

ABSTRACT

The synthetic retinoid 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437) induces apoptosis in a variety of cancer cells. Recently, we demonstrated that CD437 induces apoptosis in human non-small cell lung cancer (NSCLC) cells expressing wild-type p53 by increasing the level of the death domain-containing cell surface receptor Fas. In the present study, we investigated whether CD437 induced the expression of Fas (CD95/APO-1), a cell surface protein belonging to the tumor necrosis factor receptor superfamily, which induces apoptosis upon interaction with Fas ligand (FasL) or agonistic antibodies. We found that CD437 increased the level of Fas mRNA in a time- and concentration-dependent manner in NSCLC H460 cells. The increased Fas expression was also identified at the protein level. CD437 induced Fas expression in three NSCLC cell lines with wild-type p53 but not in six NSCLC cell lines containing mutant p53. Moreover, enhanced degradation of wild-type p53 protein in NSCLC cells expressing human papillomavirus-16 E6 oncoprotein blocked CD437-induced Fas expression. These results implicate the involvement of wild-type p53 in CD437-induced Fas expression in human NSCLC cells. CD437 did not change Fas mRNA stability, and actinomycin D abolished CD437-induced expression of Fas mRNA, suggesting that CD437 induces Fas expression at the transcriptional level. The combination of CD437 and FasL or CD437 and agonistic anti-Fas antibody caused synergistic induction of apoptosis. Furthermore, CD437 augmented Fas/Fas-L-induced apoptosis in cell lines with wild-type p53 but not in cell lines having mutant p53, indicating that a p53-dependent mechanism is also involved in this effect. Taken together, these results demonstrate that increased Fas expression may play an important role in CD437-induced, p53-dependent apoptosis in human NSCLC cells.

INTRODUCTION

Fas (also known as CD95/APO-1) is a cell surface protein belonging to the tumor necrosis factor receptor superfamily, which is expressed in a variety of normal and neoplastic cells (1–3). Triggering of Fas signaling by FasL3 or agonistic antibodies results in rapid induction of apoptosis in susceptible cells (1). Fas/FasL-induced apoptosis was initially recognized as a critical mechanism in the regulation of immunohomeostasis (1, 4). However, Fas/FasL interaction has been shown recently to play an important role in controlling tumor development, growth, and metastasis (5–8). Fas can be up-regulated upon treatment with chemotherapeutic agents (9–11) or ionizing radiation (11–13), which may mediate anticancer drug- or irradiation-induced apoptosis in a variety of cancer cells (9–13).

One mechanism of Fas expression up-regulation involves p53 (9, 12, 14). Fas expression can be transactivated by wild-type p53 through p53 binding sites in the promoter and first intron of the Fas gene (15). It is well documented that the p53 gene product is required for cells to initiate apoptosis in response to genotoxic damage induced by DNA-damaging agent, including ionizing radiation and certain cancer chemotherapeutic drugs (16). Bax is the first proapoptotic gene shown to be a direct transregulational target of p53 (17). However, Bax appears to contribute only in part to p53-mediated apoptosis (18). Because the presence of functional wild-type p53 is closely coupled with efficient induction of Fas-mediated apoptosis in many (9, 12, 13, 19–21) although not all cell types (22, 23), Fas could be an important mediator of p53-induced apoptosis. Indeed, recent study shows that Fas can mediate p53-induced apoptosis (24).

The novel synthetic CD437 is a potent inducer of apoptosis in a variety of cancer cell types including breast cancer (25), melanoma cells (26), cervical cancer (27), leukemia (28), and lung cancer (29–31) cells. Although CD437 can selectively bind to and transactivate the retinoic acid receptor γ (32), it is thought that this retinoid induces apoptosis through a unique mechanism that is independent of the RAR-mediated pathway, as was demonstrated in breast cancer cells (25) and lung cancer (31). Our previous studies demonstrate that CD437 induces apoptosis in human NSCLC cells through p53-dependent and/or -independent pathways, depending on whether cells have wild-type p53 gene (33, 34). CD437 increased the level of p53 protein and subsequently induced the expression of p53-regulated genes such as Bax, p21(WAF1/CIP1), and FasL, which eventually triggered apoptosis through induction of cytochrome c release from mitochondria and caspase-3 activation (33, 34).

Because Fas is a p53-regulated gene and CD437 induces p53-dependent apoptosis in some lung cancer cells, it is plausible to hypothesize that CD437 may also regulate Fas expression in some lung cancer cell lines with wild-type p53. To test this hypothesis, we examined the effect of CD437 on Fas expression in human NSCLC cells. In this report, we found that CD437 induced Fas expression in human NSCLC cells by a mechanism that is dependent on wild-type p53. Moreover, CD437 augmented Fas/Fas-L-mediated apoptosis. These results not only suggest an important role for Fas in CD437-induced apoptosis but also provide further support for the role of p53 in CD437-induced apoptosis in human NSCLC cells.

MATERIALS AND METHODS

Reagents. CD437, CD2325, CD271, and CD666 (32, 35) were provided by Dr. Braham Shroott (Gelderma R+D, Sophia Antipolis, France). ATRA was obtained from Dr. Werner Bollag (F. Hoffmann-La Roche, Basel, Switzerland). LG1069 (35) was provided by Dr. Richard Heyman (Ligand Pharmaceuticals, San Diego, CA). 4HPR was obtained from Dr. Ronald Lubet (National Cancer Institute, Bethesda, MD). AG193109 (35) was provided by Dr. Roshantha A. S. Chandraratana (Allergan, Irvine, CA). These retinoids were dissolved in DMSO at a concentration of 10 mM and stored in the dark at −80°C under N2 atmosphere. Stock solutions were diluted to the desired final concentrations with growth medium just before use. Act D and CHX were purchased from Sigma Chemical Co. (St. Louis, MO). Recombinant soluble human FasL was purchased from Alexis Biochemicals (San Diego, CA). Recombinant soluble human Fas:Fc chimera was purchased from R&D Sys-
temps, Inc. (Minneapolis, MN). Agonistic anti-human Fas antibody (clone CH-11) and neutralizing anti-human Fas antibody (clone ZB4) were purchased from Upstate Biotechnology (Lake Placid, NY) and MBL International Corporation (Watertown, MA), respectively.

**Cell Lines and Cell Culture.** Human NSCLC cell lines H460, A549, and H1994 that possess wild-type p53 and cell lines H596, H157, H522, Calu-1, SK-MES-1, and H1792 that have mutant or null p53 (36, 37) were either obtained from Dr. Adi Gazdar (University of Texas Southwestern Medical Center, Dallas, TX) or purchased from the American Type Cell Culture (Rockville, MD). H460 cells harboring HPV-16 E6 were provided by Dr. Wafik S. El-Deiry (University of Pennsylvania School of Medicine, Philadelphia, PA). These cells were grown in monolayer culture in a 1:1 (v/v) mixture of DMEM and Ham’s F-12 medium supplemented with 5% FBS and antibiotics at 37°C in a humidified atmosphere consisting of 5% CO2 and 95% air.

**Northern Blot Analysis.** Total cellular RNA was isolated using the TriReagent (Molecular Research Center, Inc., Cincinnati, OH). Total RNA (30 μg) was electrophoresed in a 0.66 m formaldehyde-1.2% agarose gel and then transferred to a GeneScreen nylon membrane (NEN Life Science Products, Boston, MA) and UV cross-linked. Human Fas cDNA probe (Alexis Biochemicals) and GAPDH cDNA (Ambion, Inc., Austin, TX) were labeled with [γ-32P]dCTP (ICN Pharmaceuticals, Inc., Costa Mesa, CA) to a specific activity of approximately 2 × 10^9 cpm/μg by the Prime-it II Random Primer Labeling kit (Stratagene, La Jolla, CA). After hybridization overnight at 68°C in RapidHyb buffer (Amersham Life Science, Inc., Arlington Heights, IL), the blots were washed as described previously (33) and then placed against X-ray film (Hyperfilm-MP, Amersham) for autoradiography at −80°C using double intensifying screens. Quantitation of mRNA levels was done by a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) using ImageQuant software.

**Western Blot Analysis.** Whole-cell lysates were prepared as described previously (33), and the protein concentration was determined with the Protein Assay kit (Bio-Rad, Hercules, CA). Protein (50 μg) was electrophoresed through a 10% denaturing polyacrylamide slab gel and transferred to a PVDF-PLUS membrane (MSI Micron Separations, Inc., Westborough, MA) by electroblotting. Immunoblottings for Fas, FasL, and actin were performed using mouse monoclonal antihuman Fas antibody (G254-274; PharMingen, San Diego, CA), mouse monoclonal antihuman FasL antibody (G247-4; PharMingen), and rabbit anti-actin antibody (Sigma), respectively. The blots were developed using the Renaissance Western Blot Chemiluminescence Reagent Plus (NEN Life Science Products).

**Flow Cytometric Analysis.** Direct antibody staining and flow cytometric analysis for detection of total cell and cell surface Fas were carried out. After 20 h treatment with CD437, cells were harvested by trypsinization and counted. After washing once with PBS containing 0.1% sodium azide and 1% FBS, 10^6 cells in 50 μl PBS containing 0.1% sodium azide and 1% FBS were incubated with 20 μl R-phycocerythrin-conjugated mouse antihuman Fas monoclonal antibody (DX2; PharMingen) or isotype-matched R-phycocerythrin-conjugated mouse IgGk monoclonal immunoglobulin isotype control (MOPC-21; PharMingen) for 30 min on ice. Samples were washed twice with PBS containing 0.1% sodium azide and 1% FBS, resuspended in 500 μl PBS containing 1% paraformaldehyde, and analyzed with a FACScan (Becton Dickinson, Mountain View, CA). The mean fluorescence intensity that represents antigenic density on a per cell basis was used to represent the Fas expression.

**DNA Fragmentation Assay.** Cells at density of 5000/well were plated on 96-well cell culture plates 1 day before treatment. After a 24-h treatment, DNA fragmentation was evaluated by examination of cytoplasmic histone-associated DNA fragments (mono- and oligonucleosomes) using a Cell Death Detection ELISA™ kit (Roche Molecular Biochemicals, Indianapolis, IN) according to the manufacturer’s instructions.

**RESULTS**

**CD437 Up-Regulates Fas Expression but not FasL in H460 Cells.** Because Fas is a p53-regulated gene, we first examined the effect of CD437 on the expression of Fas mRNA in H460 cells carrying wild-type p53. As shown in Fig. 1, CD437 increased the level of Fas mRNA in both time- and concentration-dependent fashions. Up-regulation of Fas mRNA by CD437 was observed as early as 3 h after treatment. The highest induction of Fas mRNA occurred 24 h (>15-fold increase) after the cells were exposed to CD437. CD437 increased the level of Fas mRNA beginning with 0.2 μM. It was reported that in some human cell lines, two species of Fas mRNA (2.7 and 1.9 kb) generated by an alternative use of two different poly(A) addition signals (38) could be detected by Northern blotting. In our study, we detected an additional weak band of ~4.5 kb after treatment with CD437. We do not know whether this extra band represents a different isoform of Fas mRNA or different splicing form of Fas mRNA. In any event, it is only a minor mRNA species.

We also detected concentration-dependent increase of Fas protein by Western blot analysis after the H460 cells were exposed to the indicated concentrations of CD437 for 20 h (Fig. 2 A). Because the functional Fas molecules are located on the surface of cells, we further analyzed the expression of cell surface Fas after treatment with CD437 by direct antibody staining and flow cytometric analysis. More than 96% of H460 cells exhibited cell surface Fas positivity after staining with anti-Fas antibody. After treatment with 1 μM CD437 for 20 h, there was an increase from 2.32 (DMSO control) to 3.66 (CD437 treatment) in mean fluorescence intensity of cell surface Fas expression (Fig. 2B). This indicates that CD437 treatment caused 66.4% increase over DMSO control in cell surface Fas expression. To find out the specificity of modulation of Fas expression by retinoids, we compared the effects of different retinoids on the expression of Fas mRNA in H460 cells. As shown in Fig. 3, Fas mRNA was up-regulated strongly by CD437 at both indicated concentrations but not by other retinoids 4-HPR, CD666, and LG1069, even at 10 μM. ATRA caused a small increase in the upper Fas transcript. These findings indicate the specificity of CD437 to induce Fas expression.

To determine whether CD437 also modulates FasL expression in H460 cells, we treated the cells with different concentrations of CD437 for 20 h and then harvested the cells for preparation of whole-cell protein lysates for Western blot analysis. Neither constitutive nor CD437 increased FasL expression were detected in H460 cells (Fig. 4).

**CD437 Up-Regulation of Fas Expression Is Dependent on Wild-Type p53.** In previous studies (33, 34), we have demonstrated that p53 plays an important role in CD437-induced apoptosis in some human NSCLC cell lines with wild-type p53. We next asked whether there was a relationship between Fas regulation and p53 status in
human NSCLC cells. Therefore, we examined the effects of CD437 on Fas expression in a group of human NSCLC cell lines with different p53 status. A 15-h treatment with 1 μM CD437 up-regulated the expression of Fas mRNA only in three cell lines (H460, H549, and H1944) with wild-type p53 but not in any of the other six cell lines (H1792, SK-MES-1, H596, H522, H157, and Calu-1) with mutant or null p53 (Fig. 5A). Even at 5 μM, CD437 failed to induce Fas mRNA expression in H1792 cells (data not shown). Furthermore, transfection of HPV-16 E6 gene, which targets p53 protein for degradation, into H460 cells not only decreased the basal level of Fas mRNA expression but also abolished the ability of CD437 to up-regulate the expression of Fas mRNA (Fig. 5B). CD2325 and to a much lesser extent CD271, two other retinoids that share structural similarity with CD437, were also able to induce Fas expression in H460 cells. Higher concentration (e.g., 5 μM) of CD271 was able to induce higher levels of Fas mRNA in H460 cells (data not shown). Like CD437, CD2325, and CD271 failed to induce Fas mRNA expression in HPV-16 E6-transfected cells. Taken together, these results clearly indicate that CD437 as well as other CD437-like retinoids up-regulate Fas expression in a p53-dependent manner in some human NSCLC cells.

**CD437-induced Fas Expression Is Independent of Nuclear Retinoid Receptors.** To determine whether nuclear retinoid receptors are involved in the effects of CD437 on Fas mRNA expression, the pan RAR-specific antagonist AGN193109 was added 30 min prior to CD437 treatment. After an additional 15-h culture, the H460 cells were subjected to total RNA extraction and Northern blot analysis. As shown in Fig. 6A, AGN193109 at 10 μM, which was 10-fold molar higher than CD437, failed to block CD437-induced increase of Fas mRNA, indicating that nuclear receptors RARs were not involved in CD437-induced Fas mRNA expression.

**CD437-induced Fas Expression Is Dependent on Transcription and Protein Synthesis.** To determine whether transcription or protein synthesis is required for the induction of Fas mRNA by CD437 in human NSCLC cells, the effects of transcriptional inhibitor Act D or protein synthesis inhibitor CHX on CD437-induced Fas mRNA were analyzed. As shown in Fig. 6A, Act D completely abolished basal level of Fas mRNA as well as induction of Fas mRNA by CD437, demonstrating a requirement of transcription for CD437-induced Fas mRNA expression. CHX alone actually increased Fas mRNA, whereas cotreatment with CD437 partially suppressed CD437-up-regulated Fas mRNA. This result indicates that protein synthesis is also required for up-regulation of Fas mRNA by CD437. Furthermore, we examined the Fas mRNA stability in the same cell line (H460) treated with and without CD437 treatment. The degradation rate of CD437-treated Fas mRNA remained similar to that of untreated control Fas mRNA, indicating that CD437 did not alter the stability of Fas mRNA (Fig. 6B).

**CD437-induced Apoptosis Requires Transcription.** Because the transcriptional inhibitor Act D suppresses induction of Fas expression by CD437, we further examined the effect of Act D on apoptosis induction by CD437. As shown in Fig. 6C, CD437-induced apoptosis was abolished by concurrent treatment of H460 cells with Act D but not by cotreatment of the cells with AGN193109, indicating that CD437-induced apoptosis is dependent on transcription but not on retinoid receptors. Because the H460 cells are extremely sensitive to toxicity of CHX, we failed to determine the effect of CHX, at doses...
that inhibit protein synthesis, on CD437-induced apoptosis (data not shown).

**CD437 Augments Fas/FasL-mediated Apoptosis.** We hypothesized that CD437 should be able to augment Fas/FasL-mediated apoptosis upon cotreatment of cells with FasL if the Fas/FasL system is involved in CD437-induced apoptosis. Therefore, we treated H460 cells with suboptimal concentration (0.2 μM) of CD437 in combination with low doses of FasL for 24 h and then analyzed apoptosis by means of an ELISA that quantitatively measures cytoplasmic histone-associated DNA fragments occurring during apoptosis. CD437 or FasL alone caused only a low level of DNA fragmentation, whereas CD437, in combination with either dose of FasL, exhibited a more than additive (presumably synergistic) induction of DNA fragmentation in H460 cells (Fig. 7A). This effect could be reproduced in another cell line (A549) with wild-type p53 but not in two other cell lines (Calu-1 and H1792) containing mutant p53 (Fig. 7B). In the presence of recombinant soluble human Fas-Fc chimera, this synergistic apoptosis-inducing effect was completely abolished (Fig. 7C). Similarly, cotreatment of H460 cells with CD437 (0.2 μM) and agonistic anti-Fas antibody (Ab-CH11) at concentrations ranging from 10 to 50 ng/ml exhibited more than additive induction of apoptosis (Fig. 7D). This effect was completely suppressed by cotreatment of the cells with neutralizing anti-Fas antibody (Ab-ZB4; Fig. 7E). Taken together, these results demonstrate that CD437 augments Fas/FasL-mediated apoptosis through a p53-dependent induction of Fas expression in human NSCLC cells.

**DISCUSSION**

Our previous studies have demonstrated that CD437 elevated the level of p53 protein and induced the expression of p53-regulated downstream genes including Killer/DR5, Bax, and p21(WAF1/CIP1), which contribute to CD437-induced apoptosis in human NSCLC cells (33, 34). In the present study, we found that CD437 up-regulated Fas expression at the mRNA level in a time- and concentration-dependent manner in H460 cells. By Western blot analysis and flow cytometric analysis, we further demonstrated that CD437 increased both total cellular Fas protein level and functional cell surface Fas level in the same cell line. Furthermore, we found that Fas up-regulation by CD437 occurred only in NSCLC cell lines with wild-type p53 but not in cell lines with mutant or null p53. The transfection of HPV-16 E6 gene, the product of which facilitates degradation of p53 protein in a ubiquitin-dependent pathway (39), into H460 cells not only diminished basal level of Fas mRNA but also abolished the ability of CD437 to up-regulate Fas expression. These results clearly indicate that CD437 causes a p53-dependent up-regulation of Fas expression in some human NSCLC cell lines with wild-type p53. To the best of our knowledge, this is the first report that CD437 up-regulates Fas expression in human cancer cells. The only report relevant to the modulation of Fas/FasL system by retinoids is that 9-cis retinoic acid inhibited activation-induced FasL up-regulation in T-cell hybridomas (40). However, we did not observe modulation of FasL expression in H460 cells, indicating that CD437 selectively induces Fas expression in human NSCLC cells.

The major biological activities of retinoids are thought to be mediated by two types of nuclear retinoid receptors, RARs and retinoid X receptors, which are members of the steroid hormone receptor gene superfamily (41). Although CD437 can selectively bind to and activate the retinoic acid receptor γ (32), it is thought that this retinoid induces apoptosis through a unique mechanism that is independent of the RAR-mediated pathway (25, 31). In this study, we noted that Fas expression was induced by CD437 but not by other retinoids such as 4HPR, ATRA, CD666, and LG1069 that have different receptor selectivities. Furthermore, we found that AGN193109, a potent pan RAR-specific antagonist, failed to block CD437-induced Fas expression. Therefore, we conclude that CD437 induces Fas expression through an RAR-independent pathway. Because AGN193109 also failed to block CD437-induced apoptosis, this indicates that a retinoid receptor independent mechanism is involved in CD437-induced apoptosis, which is agreement with our previous finding (31).

Two other retinoids, CD2325 and CD271, with RARγ selectivity
Fig. 6. CD437 up-regulates Fas expression at the transcriptional level (A and B) and requirement of transcription for CD437-induced apoptosis (C). A, the transcription inhibitor Act D and the protein synthesis inhibitor CHX, but not the RAR antagonist AGN193109, inhibit CD437-induced Fas mRNA expression in H460 cells. The cells were pretreated with 5 μg/ml Act D, 10 μg/ml CHX, or 10 μM AGN193109 for 30 min prior to 14-h cotreatment with 1 μM CD437 and Act D, CHX, or AGN193109. Total RNA preparation and Northern blot analysis were performed as described in “Materials and Methods.” Columns, relative mRNA level normalized to GAPDH. The results are plotted as described in “Materials and Methods.” A, effects of Act D and AGN193109 on CD437-induced apoptosis in H460 cells. Cells were seeded at a density of 5000/well in 96-well cell culture plates 1 day before treatment. After 8-h concurrent treatment with 2 μg/ml CD437 and the indicated concentrations of Act D or with CD437 and 20 μM AGN193109, cells were subjected to detection of DNA fragmentation using an ELISA method. Bars, SD. Columns, means of triplicate determinations.

(35) also up-regulated Fas expression in H460 cells, which was also dependent on wild-type p53 because transfection of HPV-16 E6 into the cells prevents these retinoids from inducing Fas expression. Because these three retinoids share very similar chemical structure (35) and cause p53-dependent up-regulation of Fas expression, we think that the up-regulation of Fas expression by retinoids CD2325 and CD271 is attributable to their structural similarity with CD437 rather than receptor selectivity.

It was reported that CD437 increased the levels of two p53-target genes p21 (WAF1/CIP1) mRNA and GADD45 mRNA in human breast (25) and lung cancer (42) cells, respectively. The increases in both p21 (WAF1/CIP1) and GADD45 mRNAs by CD437 were demonstrated to occur by posttranscriptional mechanisms because CD437 stabilized the mRNAs of these two genes (25, 42). In the present study, several lines of evidence suggest that CD437 may up-regulate Fas mRNA at the transcriptional level in human NSCLC cells: (a) CD437 did not alter the stability of Fas mRNA, indicating that CD437-increased Fas mRNA is not caused by a posttranscriptional mechanism through mRNA stabilization; (b) the transcription inhibitor Act D abolished up-regulation of Fas mRNA by CD437, indicating that transcription is required for CD437-increased Fas mRNA; and (c) we found that degradation of p53 protein by HPV-16 E6 abolished CD437-induced up-regulation of Fas mRNA, and up-regulation of Fas mRNA by CD437 occurred only in NSCLC cell lines with wild-type p53, indicating that Fas up-regulation is dependent on wild-type p53. Taken together, these results strongly suggest that CD437 up-regulates Fas mRNA expression at the transcriptional level in human NSCLC cells.

Our previous study has demonstrated that CD437 induces a p53-dependent apoptosis in some human NSCLC cells in which Bax and another death receptor Killer/DR5 play important roles, because targeted degradation of p53 protein by HPV-16 E6 has led to not only blockage of expression of these genes but also to suppression of subsequent induction of apoptosis by CD437 (34). In the present study, targeted degradation of p53 protein by HPV-16 E6 resulted in complete blockage of CD437-induced Fas up-regulation as well. In addition, Act D abolished not only CD437-induced Fas expression but also CD437-induced apoptosis. Therefore, the current study has identified another mediator to CD437-induced, p53-dependent apoptosis in human NSCLC cells. This study also provides supporting evidence for the role of p53 in CD437-induced apoptosis as demonstrated previously (33, 34).

Because CD437 increased the expression of functional cell surface Fas, we presumed that CD437 should be able to augment Fas/FasL-mediated apoptosis. Indeed, more than additive induction of apoptosis was observed when H460 cells, in which Fas expression was induced by CD437, were cotreated with CD437 and FasL or CD437 and agonistic anti-Fas antibody. These synergistic effects could be abolished by recombinant soluble human Fas:Fc and neutralizing anti-Fas antibody, respectively. Therefore, we conclude that CD437 augments Fas/FasL-mediated apoptosis through up-regulation of Fas expression in human NSCLC cells. Furthermore, we observed that CD437 augmented FasL-induced apoptosis in cell lines (H460 and A549) with wild-type p53, in which Fas expression was increased but not in cell lines (Calu-1 and H1792) containing mutant p53, in which Fas expression was not induced by CD437. These results indicate that CD437 augments Fas/FasL-mediated apoptosis in a p53-dependent fashion.

It has been documented that the Fas/FasL system plays a general role in induction of cytotoxicity by anticancer drugs in a variety of cells of different histotype (15). Up-regulation of Fas renders a tumor cell chemotherapy-sensitive (15). In FasL-expressing tumor cells, binding of FasL to the increased number of Fas receptors initiates the apoptotic signal in chemosensitive cells (9, 15). Furthermore, it has...
been proposed that tumor cells expressing Fas upon induction of anticancer therapy may become susceptible targets for killer cells (15). Up-regulation of Fas may target the tumor cells for elimination by the immune system using a Fas-dependent pathway. Thus, in addition to their direct cytotoxic effects, chemotherapeutic drugs sensitize tumor cells to Fas-mediated cytotoxicity and Fas-dependent immune clearance (15). In terms of NSCLC, patients with Fas-positive tumors exhibited significantly longer survival times than patients with Fas-negative carcinomas, whereas FasL did not significantly influence patient survival time (43). It was also reported that most human lung cancer cell lines and tumor tissues express functional FasL (44). Therefore, our finding that CD437 up-regulates Fas expression and augments Fas/FasL-mediated apoptosis in some human NSCLC cells may have important clinical implications for prevention and treatment of lung cancer, especially those with wild-type p53.

ACKNOWLEDGMENTS

We thank Drs. A. Gazdar and W. S. El-Deiry for providing us with some human NSCLC cell lines and HPV-16 E6-transfected H460 cells, respectively. We are grateful to Drs. B. Shroot, W. Bollag, R. Lubet, R. Heyman, and R. A. S. Chandraratna for providing us with some retnoids.

REFERENCES


Induction of Fas Expression and Augmentation of Fas/Fas Ligand-mediated Apoptosis by the Synthetic Retinoid CD437 in Human Lung Cancer Cells

Shi-Yong Sun, Ping Yue, Waun Ki Hong, et al.

Cancer Res 2000;60:6537-6543.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/22/6537

Cited articles
This article cites 43 articles, 19 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/22/6537.full#ref-list-1

Citing articles
This article has been cited by 12 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/60/22/6537.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.