The Synthetic Retinoid CD437 Selectively Induces Apoptosis in Human Lung Cancer Cells while Sparing Normal Human Lung Epithelial Cells

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ABSTRACT

The synthetic retinoid 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437) induces apoptosis in a variety of cancer cells including lung cancer cells. Our previous studies have demonstrated that cancer cells with wild-type p53 are more sensitive to CD437 than those having mutant p53, although CD437 can induce both p53-dependent and -independent apoptosis. Because normal human lung epithelial cells have wild-type p53, the question arose as to whether they are also sensitive to CD437-induced apoptosis. To address this question, we compared and contrasted the effects of CD437 on apoptosis induction and the expression of several p53-regulated apoptosis-related genes between normal human lung epithelial cells and human lung cancer cells containing wild-type p53. CD437 induced apoptosis as evidenced by apoptotic morphological changes, increased DNA fragmentation, and activation of caspase cascades in two lung cancer cell lines but not in two normal human lung epithelial cells. CD437 selectively increased the p53 protein level and concomitantly induced the expression of several p53-regulated apoptosis-related genes including Bax, Fas, DR4, and DR5 only in the two lung cancer cell lines. Furthermore, the normal lung epithelial cells, which expressed constitutively higher levels of two antiapoptotic decoy receptors DcR1 and DcR2 than lung cancer cells, exhibited an increase in the expression of these receptors after CD437 treatment, whereas no increase was detected in lung cancer cells. These results predict a differential effect of CD437 on tumor and normal cells in vivo and strongly suggest that CD437 may be a useful agent for chemoprevention and/or treatment of human cancer, especially lung cancer.

INTRODUCTION

In the United States, lung cancer is the leading cause of cancer mortality among both men and women. It has been estimated that there will be 169,500 new cases and 157,400 deaths from lung cancer in 2001 (1). Unfortunately, the severe morbidity of lung cancer and the poor 5-year relative survival rate (only 14%) have not been improved by current treatments. Therefore, intense efforts are being mounted to find effective new agents and treatments against lung cancer.

Retinoids are a class of natural and synthetic vitamin A analogues, some of which have shown potential and promise for prevention or treatment of certain types of cancer (2). One of the synthetic retinoids, CD437, has been demonstrated recently to induce apoptosis in a variety of cancer cell types including lung cancer cells (3–10). Its antitumor activity was also demonstrated in animal xenograft models, showing the potential for cancer prevention and/or treatment (4, 6).

Although CD437 can selectively bind to and transactivate the retinoic acid receptor γ (11), it is thought that this retinoid induces apoptosis through a unique mechanism that is independent of the retinoic acid receptor-mediated pathway (7, 8, 10). Our previous studies demonstrated that CD437 induces apoptosis in human lung cancer cells through p53-dependent and/or -independent pathways, depending on whether cells have the wild-type p53 gene (12, 13). CD437 increased the level of p53 protein and subsequently induced the expression of p53-regulated genes such as Bax, Fas, and DR5, which eventually triggered apoptosis through induction of cytochrome c release from mitochondria and caspase-3 activation (12–14).

Because human lung cancer cells containing wild-type p53 are more sensitive to CD437-induced apoptosis (12, 13), a concern could be raised that normal human lung epithelial cells, which have wild-type p53, may also be sensitive to CD437 treatment. This study was designed to address this question by comparing and contrasting the effects of CD437 on apoptosis induction and on the expression of several p53-regulated, apoptosis-related genes between human lung cancer cells having wild-type p53 and normal human lung epithelial cells. We found that CD437 selectively induced apoptosis in human lung cancer cells while sparing normal human lung epithelial cells. In addition, CD437 exhibited differential modulation of several p53-regulated, apoptosis-related genes between the two types of cell. Our current results warrant additional studies on the potential of CD437 as a preventive and/or therapeutic agent for lung cancer.

MATERIALS AND METHODS

Reagents. CD437 (11) was provided by Galderma R&D (Sophia Antipolis, France). It was dissolved in DMSO at a concentration of 10 mM and stored in the dark at −80°C under N₂ atmosphere. Stock solution was diluted to desired final concentrations with growth medium just before use.

Cell Lines and Cell Culture. Human lung cancer cell lines H460 (large cell carcinoma) and H292 (mucoepidermoid carcinoma) cells were purchased from American Type Cell Culture (Rockville, MD). These cells were grown in monolayer culture in a 1:1 (v/v) mixture of DMEM and Ham’s F12 medium supplemented with 5% FBS and antibiotics at 37°C in a humidified atmosphere consisting of 5% CO₂ and 95% air. Under such a culture condition, the doubling times for H460 and H292 are 23 and 27 h, respectively. Human NHBE cells and SAECs, which were derived from human lung bronchus and the small airway of healthy donors, respectively, were purchased from Clonetics (San Diego, CA) and grown in serum-free Bronchial Epithelial Cell Growth Medium Bullet kit (Clonetics) at 37°C in a humidified atmosphere consisting of 5% CO₂ and 95% air. The doubling times for NHBE cells and SAECs are 17–31 h and 18–26 h, respectively (provided by Clonetics). In some experiments, normal cells were grown in medium supplemented with serum and cancer cells in serum-free medium.

Growth Inhibition Assay. Cells were seeded in 96-well tissue culture plates, and after 24 h, they were treated with different concentrations of CD437. Control cultures received the same amount of DMSO as did the treated cultures. After 24-h treatment, cell numbers were estimated by the SRB assay, and growth inhibition was calculated as described previously (15).

DNA Fragmentation Assay. Cells were plated in 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 ELISAPlus kit.
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Northern Blot Analysis. Procedures for preparation of total cellular RNA and Northern blotting were performed as described previously (15). Thirty µg of total RNA were loaded in each lane. Human Dr5 CDNA was obtained from Dr. W. S. El-Deiry (University of Pennsylvania School of Medicine, Philadelphia, PA). The GST-CIP plasmid containing human p21(WAF1) cDNA was purchased from the American Type Culture Collection. Human Bax cDNA was provided by Dr. S. J. Korsmeyer (Washington University School of Medical, Saint Louis, MO). Human Fas, DR4, DcR1, and DcR2 cDNAs were purchased from Alexis Biochemicals (San Diego, CA). Human glyceraldehyde-3-phosphate dehydrogenase cDNA was purchased from Ambion, Inc. (Austin, TX).

Western Blot Analysis. Whole cell lysates were prepared as described previously (12), and the protein concentration was determined using a Protein Assay kit (Bio-Rad, Hercules, CA). Cell lysates (50 µg) were electrophoresed through 7.5–12% denaturing polyacrylamide slab gels and transferred to a PROTRAN nitrocellulose transfer and immobilization membrane (Schleicher & Schuell, Inc., Keene, NH) by electroblotting. The blots were probed or reprobed with the antibodies, and then antibody binding was detected using the enhanced chemiluminescence (ECL) system (Amersham, Arlington Heights, IL) according to the manufacturer’s protocol. Mouse monoclonal anti-caspase-3 (clone 31A1067) was purchased from IMGENEX (San Diego, CA). Rabbit polyclonal anti-caspase-9, anti-caspase-6, anti-lamin A, and anti-PARP antibodies were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Mouse monoclonal anti-caspase-8 (clone 5F7) and rabbit polyclonal anti-DFF45 antibodies were purchased from Upstate Biotechnology (Lake Placid, NY). Rabbit polyclonal anti-Bid antibody was purchased from Trevigen (Gaithersburg, MD). Mouse monoclonal anti-p53 antibody (Ab-6) was purchased from Oncogene Research Products (Boston, MA). Mouse monoclonal anti-Bcl-2 (100) antibody was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Rabbit polyclonal anti-β-actin antibody was purchased from Sigma Chemical Co. (St. Louis, MO).

RESULTS

CD437 Selectively Induces Apoptosis in Human Lung Cancer Cells while Sparing Normal Human Lung Epithelial Cells. In this study, we compared the responses of two types of normal human lung epithelial cells, NHBE cells and SAECs, which were derived from human lung bronchus and the small airway of healthy donors, respectively, with two lung cancer cell lines H460 and H292 to CD437 treatment. The optimal culture conditions for NHBE cells and SAECs are serum-free medium, whereas lung cancer cells grow in the presence of 5% serum. We found that serum can affect the effects of CD437 on cell growth and death. Specifically, cells become super sensitive to CD437 under serum-free culture conditions (data not shown). Therefore, we examined the effects of CD437 on the growth and apoptosis of these cells both in the absence of serum (conditions favorable for normal epithelial cell growth) and in the presence of serum (conditions favorable for cancer cell growth). In the presence of 5% serum, CD437 (0.2–1 µM for 24-h treatment) inhibited cell growth by 30–80% in both lung cancer cell lines (Fig. 1B), whereas it showed weak (<30% at 1 µM) or no growth inhibition in both normal lung epithelial cells (Fig. 1C). In the absence of serum, lung cancer cells became very sensitive to CD437. As shown in Fig. 1D, even at 0.2 µM CD437 inhibited the growth of both cell lines by >60 and >25% at 0.05 µM. The concentration at which CD437 inhibited cell growth could be reduced to 0.05 µM. In comparison, normal lung epithelial cells were still less responsive to CD437. The maximum growth inhibition by CD437 (at 1 µM) was <20% in both NHBE cells and SAECs (Fig. 1A). We do not know how serum attenuates the effect of CD437 on cell growth. It is possible that protein (i.e., albumin) in serum binds CD437 and thereby decreases the free concentration of CD437 with access to the cells. Under normal culture conditions, NHBE cells and SAECs have doubling times of 17–31 h and 18–26 h, respectively, whereas H460 and H292 cells have doubling times of 23 and 27 h, respectively. Therefore, the growth rates of normal and malignant lung epithelial cells are comparable. In the absence of the serum, the growth of cancer cells slows down. The doubling times for H460 and H292 under a serum-free culture condition are about 29 and 35 h, respectively. Because we treated cells for 24 h, which allows cells to divide only once or less than once, the effects of CD437 on the growth of lung cancer cells are unlikely attributable to blockage of cell proliferation, rather than to cell killing (e.g., apoptosis). Indeed, we noted that, after exposure to CD437, lung cancer cells underwent morphological changes characteristic of apoptosis, such as rounding, detachment, and floating, whereas normal lung epithelial cells did not change their morphology but showed only a slight decrease in cell number (Fig. 2A). This suggests that CD437 may induce apoptosis selectively in lung cancer cells but not in normal lung epithelial cells. Under the serum-free condition, CD437 even at 0.2 µM was able to cause the majority of cancer cells to detach and float (Fig. 2B). In contrast, both normal lung epithelial cells remained attached even after prolonged treatment (up to 3 days) with 1 µM CD437 (data not shown). Therefore, it appears that growth rate is not a determinant of cell sensitivity to CD437 treatment.

Next, we compared and contrasted the effects of CD437 on apoptosis induction between normal lung epithelial cells and lung cancer cells by evaluating the formation of histone-associated DNA fragments occurring during apoptosis. CD437 induced a concentration-dependent increase in DNA fragments in both lung cancer cell lines (Fig. 3B). Similar to the growth-inhibitory effects, serum-free culture conditions have made cancer cells more sensitive to CD437-induced apoptosis. Under such conditions, 0.2 µM or even 0.05 µM CD437 increased DNA fragmentation in both lung cancer cell lines (Fig. 3D). In contrast, even at 1 µM CD437 did not increase DNA fragmentation in either of the two normal human lung epithelial cells both in the presence (Fig. 3A) or the absence (Fig. 3C) of serum. These results indicate that CD437 induces apoptosis only in lung cancer cells but not in normal lung epithelial cells.

Furthermore, we compared the effects of CD437 on the activa-
tion of the caspase cascades, which is the biological hallmark of apoptosis (16, 17), between normal lung epithelial cells and lung cancer cells by Western blot analysis. There are two types of caspases: upstream caspases called initiator caspases (e.g., caspase-8, -9, and -10) and downstream caspases known as effector or executioner caspases (e.g., caspase-3, -6, and -7; Refs. 16, 17). Activation of these caspases was detected by Western blot analysis as a decrease of procaspase forms and/or appearance of cleaved bands of procaspases as well as by increased cleavage of PARP, DFF45, and lamin proteins in lung cancer H460 cells. In the absence of serum, similar results could be gained even when the cells were treated with lower concentrations of CD437 (0.2 and 0.5 µM) for a shorter period of treatment (Fig. 4B). We did not observe any effects of CD437 (up to 1 µM) on caspase activation and cleavage of their substrates such as PARP, DFF45, and lamin in NHBE cells, which were cultured in the serum-free medium (Fig. 4). These results again indicate that CD437 induces apoptotic cell death selectively in lung cancer cells but not in normal lung epithelial cells.

CD437 Exhibits Differential Effects on the Expression of Several p53-regulated Apoptotic Genes between Human Lung Cancer Cells and Normal Human Lung Epithelial Cells. Our previous studies have demonstrated that p53-dependent induction of apoptosis and growth arrest by CD437 in human lung cancer cells involves p53 activation and up-regulation of its downstream-regulated genes including p21, Bax, DR5, and Fas (12–14). To gain further insights into the mechanism by which CD437 selectively induces apoptotic cell death in lung cancer cells while sparing normal human lung epithelial cells, we compared the effects of CD437 on p53 protein level and the expression levels of several p53-regulated, apoptosis-related genes between lung cancer cells and normal lung epithelial cells. We found that CD437 remarkably increased the level of p53 protein in both lung cancer cell lines (Fig. 5C). There was a slight increase in p53 protein level in NHBE cells but not in SAECS after exposure to CD437 (Fig. 5C). Moreover, CD437 induced the expression of Bax and DR5 in both H460 and H292 lung cancer cell lines but not in NHBE and

Fig. 2. Differential morphological changes induced by CD437 between normal (NHBE) and lung cancer (H460) cells. Cells were seeded in 10-cm diameter dishes and treated with the indicated concentrations of CD437 under optimal culture conditions for NHBE (without serum) and H460 (with serum; A) or suboptimal culture condition for H460 (without serum; B). Photographs were taken after a 24-h treatment using a Nikon microscope. ×100.

Fig. 3. CD437 induces apoptosis in human lung cancer cells (B and D) but not in normal lung epithelial cells (A and C). Cells were seeded in 96-well plates and treated with the indicated concentrations of CD437 for 24 h in the presence (B and C) or absence (A and D) of serum. DNA fragmentation was determined using an ELISA method as described in “Materials and Methods.” Columns, means of triplicate determinations; bars, SD.
SAEC normal lung epithelial cells. CD437 strongly induced the expression of Fas and DR4 in the both lung cancer cell lines but only slightly (NHBE) or not at all (SAEC) in normal lung epithelial cells. In contrast, p21 (WAF1) expression was induced by CD437 in both lung cancer cells and normal lung epithelial cells, although its induction levels in normal cells were much lower than that in cancer cells (Fig. 5A). Interestingly, the basal mRNA levels of DcR1 and DcR2, two decoy receptors for the death ligand TRAIL (18), which antagonize its action, were very low and were also not inducible (except for a 7.0-kb transcript) by CD437 in either cancer cell line. However, the basal levels of DcR1 (particularly the 4.0-kb transcript) and DcR2 were higher in both normal lung epithelial cells than in cancer cells. DcR1 mRNA expression was induced by CD437 in both NHBE cells and SAECs, whereas DcR2 was induced only in NHBE cells (Fig. 5B). DcR2 expression was not modulated by CD437 in SAECs, but its basal level in SAECs was much higher than in NHBE cells, as was the case for DcR1 (Fig. 5B).

Resistance of Normal Human Lung Epithelial Cells to CD437-induced Apoptosis Is Not Related to the Expression Level of Bcl-2 or Bcl-XI. Because Bcl-2 or Bcl-XI expression is related to resistance to apoptosis (19), we wondered whether there was any relationship between resistance of normal lung epithelial cells to CD437-induced apoptosis and the level of Bcl-2 or Bcl-XI. Therefore, we compared the expression levels of Bcl-2 and Bcl-XI in the absence and presence of CD437 between lung cancer cells and normal lung epithelial cells. As shown in Fig. 6A, NHBE cells and SAECs expressed very low basal levels of Bcl-2 and Bcl-XI compared with H460 and H292 cells, yet the cancer cells were sensitive to CD437, whereas the normal cells were resistant. We found no evidence that CD437 modulated the level of Bcl-2 or Bcl-XI in either of the cell lines. Therefore, it appears that Bcl-2 or Bcl-XI expression level does not account for the resistance of normal lung epithelial cells to CD437-induced apoptosis. To further assess the effect of Bcl-2 on the action of CD437, we compared the effects of CD437 on the growth of H460 transfectants with different expression levels of exogenous Bcl-2. As shown in Fig. 6B, two Bcl-2-transfected H460 cell lines H460-Bcl2-6 and H460-Bcl2-8 were not less sensitive to CD437 than vector-transfected control cells H460-Neo. In fact, they were even more sensitive to CD437 treatment than H460-Neo. The higher sensitivity of Bcl-2 transfected to CD437 is probably related to clonal variations because H460-Bcl2-8, which expresses a lower level of Bcl-2, was even more sensitive than H460-Bcl2-6 to CD437 treatment, which expresses higher level of Bcl-2 (Fig. 6B). Nevertheless, overexpression of exogenous Bcl-2 did not make cells more resistant to the growth-inhibitory effects of CD437. This result clearly indicates that CD437 induces apoptosis independent of Bcl-2 expression levels, at least in lung cancer cells.
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**DISCUSSION**

One of the critical questions related to potential application of proapoptotic agents is whether they distinguish between normal and malignant cells. In the present study, we compared and contrasted the effects of the synthetic retinoid CD437 on apoptosis induction in human lung cancer cells containing wild-type p53 and normal human lung epithelial cells. As demonstrated previously (12, 13), the two lung cancer cell lines underwent rapid apoptosis, evidenced by the typical morphological changes, increased DNA fragmentation, and activation of the caspase cascades after exposure to CD437. Using the same criteria for evaluation of apoptosis, we found that CD437 did not induce apoptotic cell death in two types of normal human lung epithelial cells derived from the bronchus and the small airway, respectively. Thus, it is apparent that CD437 selectively induces apoptotic cell death of lung cancer cells while sparing normal lung epithelial cells. This effect is not limited to lung epithelial cells, because in a recent study from our group (20), CD437 induced apoptosis in malignant human epidermal keratinocytes but not in their normal counterparts, although it arrested growth of the latter cells. Because CD437 also induces p53-independent apoptotic cell death in various types of cancer cells including lung cancer cells (3–10), we suggest that CD437 should be considered a strong candidate for prevention and/or treatment of human lung cancer as well as other types of cancer.

Our previous studies have shown that CD437 activated caspase-3 and increased PARP cleavage, whereas pan caspase inhibitor z-Val-Ala-Asp-fluoromethyl ketone and caspase-3 inhibitor z-Asp-Glu-Val-Asp-fluoromethylketone were able to abrogate CD437-induced apoptosis, demonstrating that activation of caspases including caspase-3 is required for CD437-induced apoptosis in human lung cancer cells (12, 13). In the present study, we extended our findings by demonstrating that CD437 also activated caspase-6 and increased cleavage of lamin and DFF45 in human lung cancer cells. Moreover, we found that CD437 activated both caspase-8 and caspase-9 in lung cancer cells. Our previous study has demonstrated that cytochrome c release from mitochondria is also required for CD437-induced apoptosis in lung cancer cells (13). Considering that CD437 up-regulated the expression of the death receptors Fas, DR4, and DR5, which are linked to caspase-8 activation, and also increased the expression of Bax, which facilitates cytochrome c release from mitochondria, it is very likely that CD437 induces apoptosis through activation of both death receptors/caspase-8- and mitochondria/caspase-9-mediated apoptotic pathways at least in lung cancer cells.

Our previous studies have demonstrated that CD437 elevated the level of p53 protein and induced the expression of p53-regulated downstream genes including DR5, Bax, Fas, and p21(WAF1/CIP1), which contribute to CD437-induced apoptosis and growth arrest in human NSCLC cells (12–14). In the present study, we could reproduce these findings in two lung cancer cell lines with wild-type p53. DR4 was found recently to be a p53-regulated gene by us (21) and others (22). It could be induced by CD437 in a p53-dependent manner in human lung cancer cells and in a p53-independent manner in human prostate cancer cells (23). Therefore, it is not surprising that CD437 induced DR4 expression in both lung cancer cell lines in the present study. Importantly, CD437 almost failed to elevate the p53 protein level and induce the expression of the above p53-regulated genes in the two normal lung epithelial cells, which are supposed to have wild-type and functional p53. Because an increase in the level of p53 and the up-regulation of its signaling pathways are critical for high effectiveness of CD437 to induce apoptosis in human lung cancer cells containing wild-type p53 (13), our current findings that CD437 failed to activate p53 and its signaling pathways and to induce apoptosis in normal human lung epithelial cells are very important.

In future studies, we hope to understand the mechanisms underlying the differential effects of CD437 on activation of p53 signaling pathways and induction of apoptosis between normal and malignant lung cells. Such an understanding will allow us to design and synthesize more effective molecules targeting selective killing of cancer cells while sparing normal tissues.

The decoy receptors DcR1 and DcR2, which contain either no cytoplasmic death domain or a truncated death domain, can bind TRAIL and thereby act as antagonists to protect cells from death receptor-mediated apoptosis (18). Recently, both DcR1 and DcR2 were reported to be p53-regulated genes (24, 25).Very interestingly, we found that the basal levels of DcR1 and DcR2 were undetectable and largely not inducible by CD437 in the two tested lung cancer cell lines, whereas normal lung epithelial cells expressed higher basal levels of the decoy receptors, which were inducible by CD437. This is consistent with the previous finding that death receptors DR4 and DR5 are expressed preferentially in many normal tissues (18). It is tempting to suggest that the failure of CD437 to induce apoptosis in normal lung epithelial cells may be associated with its failure to up-regulate the expression of several proapoptotic genes including Bax, Fas, DR4, and DR5 complemented by its ability to induce expression of anti-apoptotic genes DcR1 and DcR2 in these cells. Our ongoing study is investigating whether overexpression of DcR1 or DcR2 exerts any

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4 S-Y. Sun, P. Yue, and R. Lotan, unpublished observations.
effect on CD437-induced apoptosis in human lung cancer cells, which may provide insight into the mechanism underlying resistance of normal cells to CD437.

The current findings that CD437 has differential effects on the expression of death and decoy receptors in lung cancer cells versus normal lung epithelial cells provide a reasonable explanation for the selective augmentation of apoptosis induced by the combination of CD437 and TRAIL in lung cancer cells, which had been reported recently by us (26). CD437 shares certain similar features with TRAIL in inducing apoptosis in malignant versus normal cells. Therefore, the clinical potential of the combination of CD437 and TRAIL should be investigated in the future.

Bcl-2 and Bcl-Xl play important roles in negative regulation of apoptosis (19, 27) and are associated with chemoresistance (28, 29). Because normal lung epithelial cells were resistant to CD437-induced apoptosis, we wondered whether they express higher levels of Bcl-2 or Bcl-Xl than tumor cells that lead to resistance to CD437. However, we found that the two normal lung epithelial cells had very low levels of Bcl-2 and Bcl-Xl in comparison with the two lung cancer cell lines. CD437 did not alter the level of either Bcl-2 or Bcl-Xl in both normal and cancer cells. Overexpression of Bcl-2 inhibited CD437-induced apoptosis in the T-cell lymphoma cell line Molt-4 (30) but not in the leukemia cell line HL-60 (31), which were all conducted in non-solid tumor cell types. In our study, we found that overexpression of Bcl-2 in the lung cancer cell line H460 failed to protect cells from the action of CD437. Our results differ from the findings with T-cell lymphoma cell line (30) but agree with the findings with HL-60 leukemia cells (31). This actually is the first study to examine the role of Bcl-2 overexpression in CD437’s cytotoxic effect in solid tumor cells. Taken together, we conclude that Bcl-2 is unlikely to play a role in CD437-induced apoptosis in lung cancer cells, and its expression level cannot account for resistance of normal lung epithelial cells to CD437.

It should be pointed out that CD437 slightly decreased the number of normal lung epithelial cells without inducing cell killing. By analysis of cell cycle distribution, we found an increase in G1 phase, which is in agreement with the finding in normal epidermal keratinocytes (20). p21(WAF1) is an established regulator of G1 phase, which is in agreement with the finding in normal epidermal keratinocytes (20). In the present study, we found that CD437 also up-regulated p21(WAF1) expression in the two normal lung epithelial cells, albeit to a less extent than in lung cancer cells, which is correlated with its weak effect on growth arrest in normal cells. Therefore, it is likely that CD437 induces p21(WAF1) expression that leads to G1 arrest in normal lung epithelial cells.

In summary, the present study provides evidence that CD437 selectively induces apoptotic cell death in human lung cancer cells while sparing normal human lung epithelial cells. This selectivity of CD437 may be related to the following findings: (a) its ability to induce the expression of several proapoptotic genes including Bax, Fas, DR4, and DR5 in lung cancer cells but not in normal lung epithelial cells; and (b) its ability to induce the expression of antiapoptotic genes Dr51 and Dr52 in normal cells but not in cancer cells. This study together with other published results indicates the potential of CD437 alone or in combination with TRAIL for prevention and/or treatment of human cancer including lung cancer.

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