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Dual Mechanisms of Action of the Retinoid CD437: Nuclear Retinoic Acid Receptor-Mediated Suppression of Squamous Differentiation and Receptor-Independent Induction of Apoptosis in UMSCC22B Human Head and Neck Squamous Cell Carcinoma Cells

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ABSTRACT

The synthetic retinoid 6-[3-(adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437), which can bind to and activate the nuclear retinoic acid receptors β and γ (RARβ/γ), is a potent inducer of apoptosis in various cancer cell lines. However, this effect was reported to be independent of RARs. In this study, we compared and contrasted the potencies and mechanisms of action of CD437 and several other receptor-selective retinoids in induction of apoptosis and modulation of squamous differentiation in UMSCC22B human head and neck squamous cell carcinoma cell line. CD437 and the structurally related retinoid CD2325 exhibited almost equal potency in inducing apoptosis, whereas several other retinoids failed to induce apoptosis. The RAR-specific pan antagonist AGN193109 failed to suppress CD437-induced apoptosis, indicating that the induction of apoptosis by CD437 was RAR-independent. *c-Fos* expression was induced by CD437 and CD2325 that induced apoptosis in the cell line but not by other retinoids that failed to induce apoptosis, suggesting a role for c-Fos in CD437-induced apoptosis. At low concentration (0.01 μM), CD437 shared with several other receptor-selective retinoids the ability to suppress the mRNA levels of the squamous differentiation markers Spr1, involucrin, and cytokeratin 1. This effect of CD437 could be blocked by AGN193109. We conclude that CD437 can exert its effects in UMSCC22B human human head and neck squamous cell carcinoma cells by at least two mechanisms: RAR-mediated suppression of squamous differentiation and RAR-independent induction of apoptosis.

It has been estimated that there will be 30,200 new cases and 7,800 deaths from head and neck cancers in the United States in 2000 (Greenlee et al., 2000). The morbidity and mortality from head and neck cancer still remain a significant problem. Therefore, new approaches for the prevention and treatment of head and neck cancers have being extensively explored. Retinoids have shown promising results in suppressing oral premalignant lesions (e.g., leukoplakia) and in decreasing the incidence of second primary tumors in patients who had been treated earlier for primary head and neck cancers (Lotan, 1996; Hong and Sporn, 1997; Lippman et al., 1998). However, the long-term use and the realization of the full potential of the few retinoids that had been tested as chemopreventive agents were hampered by their undesirable systemic side effects. Therefore, the identification and development of new retinoids with a more favorable therapeutic index and with reduced side effects is being pursued.

Most of the biological activities of retinoids are thought to be mediated by two types of nuclear retinoid receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs) that are members of the steroid hormone-receptor gene superfamily (Chambon, 1996). Various synthetic retinoids having specific receptor selectivity toward individual RAR or RXR subtypes (α, β, and γ), or both RARs and RXRs have

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ABBREVIATIONS: RAR, retinoic acid receptor; RXR, retinoid X receptors; HNSCC, head and neck squamous cell carcinoma; CD437, 6-[3-(adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid; ATRA, all-*trans*-retinoic acid; CK1, cytokeratin 1; Spr1, small proline-rich protein gene; ELISA, enzyme-linked immunosorbent assay.

been identified (Sun et al., 1997b). Recently, we screened 38 receptor-selective retinoids for their ability to inhibit the growth of human non-small cell lung cancer and head and neck squamous cell carcinoma (HNSCC) cells and identified some potent retinoids, including 6-[3-(adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437) (Sun et al., 1997b, 2000).

CD437 was found to be a potent inducer of apoptosis in human lung (Sun et al., 1997a, 1999b; Adachi et al., 1998; Li et al., 1998), cervical (Oridate et al., 1997), and breast (Shao et al., 1995) cancer cells, as well as in melanoma (Schadendorf et al., 1996) and leukemia (Hsu et al., 1997; Gianni and de The, 1999; Mologni et al., 1999) cells. CD437 was reported to transactivate both RARβ and RARγ receptors (Bernard et al., 1992; Dawson et al., 1998). However, the apoptosis induced by CD437 in human cancer cells has been found to be independent of retinoid receptors (Shao et al., 1995; Hsu et al., 1997; Sun et al., 1997a).

In this study, we demonstrate the existence of two types of mechanisms by which CD437 exerts its biological effects on UMSCC22B HNSCC cells: a receptor-independent induction of apoptosis and a receptor-dependent suppression of squamous differentiation. This is the first report that CD437 can change cell phenotype through an RAR-mediated pathway in human cancer cells.

Materials and Methods

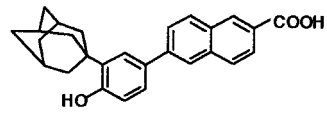
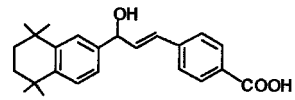
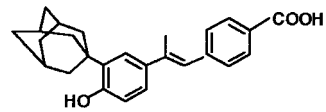
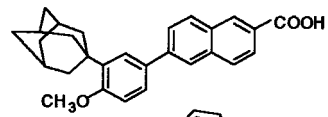
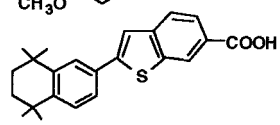
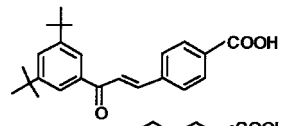
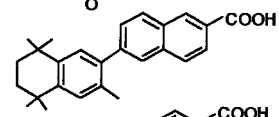
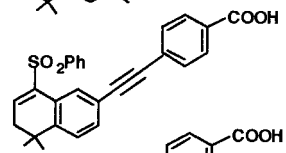
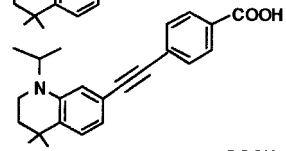
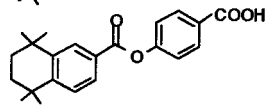
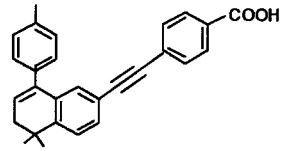
Cell Lines and Cell Culture. Human HNSCC cell lines UMSCC14B, UMSCC17B, and UMSCC22B were obtained from Dr. T. Carey (University of Michigan, Ann Arbor, MI). SqCC/Y1 was provided by Dr. M. Reiss (Yale University, New Haven, CT). TR146 was obtained from Dr. A. Balm (The Netherlands Cancer Institute, Amsterdam, The Netherlands). 183A, MDA886Ln, and 1483 cell lines were provided by Dr. P. G. Sacks (Memorial Sloan-Kettering Cancer Center, New York, NY). These cell lines were grown in monolayer culture in a 1:1 (v/v) mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium containing 5% regular fetal bovine serum and antibiotics at 37°C in a humidified atmosphere composed of 95% air and 5% CO_2.

Retinoids. CD270, CD271, CD2325, and CD437 were provided by Dr. B. Shroot (Galderma R+D, Sophia Antipolis, France). SR3985 was obtained from Dr. M. I. Dawson (SRI International, Menlo Park, CA). Ch55 was obtained from Dr. K. Shudo (Tokyo University, Tokyo, Japan). AGN193273, AGN190521, AGN193109 and AGN193078 were synthesized by Allergan (Irvine, CA). All-*trans*-retinoic acid (ATRA) was obtained from Dr. W. Bollag (F. Hoffmann-La Roche, Basel, Switzerland). The chemical structures and receptor selectivities of these retinoids are presented in Table 1. Retinoids were dissolved in dimethyl sulfoxide at a concentration of 10 mM and stored under N_2 in the dark at −80°C. Stock solutions were diluted to the appropriate concentrations with serum-containing growth medium just before use.

RNA Purification and Northern Blotting. Total RNA preparation and Northern blotting were performed as described previously (Sun et al., 1997b). Human small proline-rich protein gene (*Spr1*) cDNA in Bluescript vector was described before (Tesfaigzi et al., 1993). Human cytokeratin 1 (*CK1*) cDNA in pGEM3 vector (Steiner et al., 1985) was obtained from Dr. D. Roop (Baylor College of Medicine, Houston, TX). Human *involucrin* cDNA in pBRI-2 vector (Eckert and Green, 1986) was provided by Dr. R. Eckert (Case Western Reserve University School of Medicine, Cleveland, OH). Glyceraldehyde-3-phosphate dehydrogenase cDNA was purchased from Ambion (Austin, TX). pSVc-Myc-1 plasmid containing mouse *c-Myc* cDNA and human *c-Fos* cDNA in pBK28 were obtained from Dr. P. Chiao (The University of Texas M. D. Anderson Cancer Center, Houston, TX). JAC.1 plasmid containing mouse *c-Jun* cDNA was purchased from the American Type Culture Collection (Rockville, MD). pCR-Killer-Race-6 plasmid containing human *Killer/DR5* cDNA was provided by Dr. W. S. El-Deiry (University of Pennsylvania School of Medicine, Philadelphia, PA). Human *Bax* cDNA in pSFV-neo vector provided by Dr. S. J. Korsmeyer (Washington University School of Medical, St. Louis, MO).

Growth Inhibition Assay. Cells were seeded at a density of 2000 to 5000/well in 96-well tissue culture plates 1 day before treatment.

TABLE 1
Chemical structures and receptor selectivities of the retinoids used in this study

Retinoid	Structure	RAR Selectivity
CD437		β/γ [a]
CD666		γ
CD2325		γ
CD271		β/γ
CD270		β/γ
Ch55		α/β/γ
SR3985		β/γ
AGN193273		β/γ
AGN193078		β/γ
AGN190521		α/β/γ
AGN193109		α/β/γ [b]

[a] Dawson et al. (1998).
[b] Antagonist.

After treatment with retinoids, cell number was estimated by the sulforhodamine B assay (Sun et al., 1997b) and growth inhibition was calculated as previously described (Sun et al., 1997b).

DNA Fragmentation Assay. Cells were plated on 10-cm-diameter dishes 1 day before treatment. After 24-h treatment with retinoids, cells were harvested by trypsinization and counted. Cell Death Detection Enzyme-Linked Immunosorbent Assay (ELISA)^{Plus} kit (Boehringer Mannheim, Indianapolis, IN) was used according to the manufacturer's protocol to detect cytoplasmic histone-associated DNA fragments (mono- and oligonucleosomes) occurring during apoptosis. In addition, APO-DIRECT TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) kit (Phoenix Flow Systems, Inc., San Diego, CA) was used following the manufacturer's protocol to determine DNA fragments with 3′-hydroxyl ends.

Measurement of Caspase-3 Activity. Cells were plated onto 10-cm-diameter dishes 1 day before treatment. After a 24-h treatment with retinoids, cells were harvested by trypsinization and counted. Caspase-3 activity was measured as described by Kagaya et al. (1997). Briefly, 1×10^6 cells were lysed for 20 min on ice in 50 μl of lysis buffer containing 10 mM HEPES-KOH (pH 7.4), 2 mM EDTA, 0.1% 3-[(3-chloramidopropyl)dimethylaminonie]-1-propanesulfonate, 1 mM phenylmethylsulfonyl fluoride, and 5 mM dithiothreitol. After the lysates were transferred to a flat bottom 96-well culture plate, 50 μl of reaction buffer containing 40 mM HEPES-KOH (pH7.4), 20% glycerol (v/v), 1 mM phenylmethylsulfonyl fluoride, 4 mM dithiothreitol, and 50 μM fluorogenic substrate Ac-DEVD-AFC (*N*-acetyl-Asp-Glu-Val-Asp-AFC) (Biomol, Plymouth Meeting, PA) was added to each well and the plate was incubated at 37°C for 1 h. The fluorescence intensity was measured at 500 nm after excitation at 400 nm by using a CytoFluor Multi-Well Plate Reader Series 400 (PerSeptive Biosystems, Framingham, MA). An increase in fluorescence intensity was used to represent relative caspase-3 activity.

Results

Induction of Apoptosis by CD437. Several retinoids, which were previously found to be effective in inhibiting the growth of UMSCC22B cells in a 6-day assay (Sun et al., 2000), were examined for their ability to induce apoptosis in UMSCC22B cells by analysis of caspase-3 activation and DNA fragmentation, which are important events during apoptosis (Bortner et al., 1995; Patel et al., 1996). As shown in Fig. 1A. the retinoids tested inhibited cell growth by 55 to 98% at 1 μM. However, only CD437 and CD2325 induced both caspase-3 activity and DNA fragmentation (B and C).

CD437-Induced Apoptosis Is Not Inhibited by an RAR-Selective Antagonist. If CD437-induced apoptosis involves RAR-signaling pathway, then cotreatment of cells with RAR-selective antagonist should suppress apoptosis induction by CD437. However, the pan RAR-specific antagonist AGN193109 (10 μM) failed to block CD437 (1 μM)-induced apoptosis in UMSCC22B cells (Fig. 2), indicating that CD437-induced apoptosis is independent of RARs in the UMSCC22B cells.

Modulation of Expression of Apoptosis-Related Genes by CD437. We examined the effects of CD437 on the expression in UMSCC22B of several apoptosis-related genes, which have been implicated in CD437-induced apoptosis in other types of cancer cells (Shao et al., 1995; Schadendorf et al., 1996; Adachi et al., 1998; Li et al., 1998; Sun et al., 1999a,b,c). We did not detect modulation by CD437 of the levels of *Killer/DR5*, *Bax*, *c-Myc*, and *c-Jun* mRNAs in this cell line after either a 7- or a 15-h treatment (data not shown). In contrast, *c-Fos* expression was increased by CD437 and CD2325 that induced apoptosis but not by other retinoids that failed to induce apoptosis (Fig. 3A). This result suggests that c-Fos may contribute to CD437-induced apoptosis in UMSCC22B cells. AGN193109 failed to block the increase in c-Fos level induced by CD437 (Fig. 3B), indicating

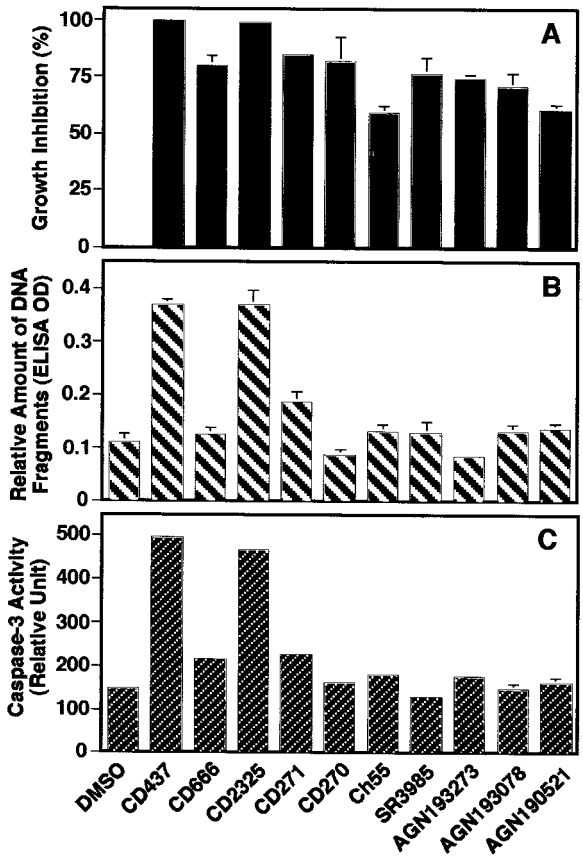


Fig. 1. Effects of CD437 and other retinoids on cell growth (A), induction of DNA fragmentation (B), and caspase-3 activation (C) in UMSCC22B cells. The cells were treated with 1 μM each of the retinoids for 6 days (A) or 24 h (B and C). The assays for growth inhibition, DNA fragmentation (ELISA), and caspase-3 activity were described, respectively, under *Materials and Methods*. Columns, means of quadruplicate (A) or triplicate (B and C) determinations; bars, S.D.

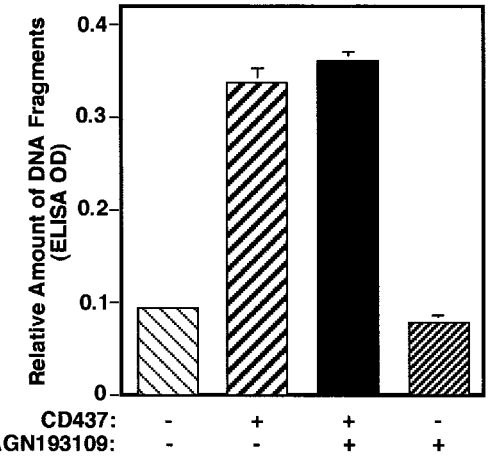


Fig. 2. Effects of the RAR pan antagonist AGN193109 on the induction of apoptosis by CD437 in UMSCC22B cells. The cells were treated concurrently with 10 μM AGN193109 and 1 μM CD437 for 24 h. DNA fragmentation was estimated by ELISA as described under *Materials and Methods*. Columns, means of triplicate determinations; bars, S.D.

that this effect of CD437-induced *c-Fos* expression is also independent of RARs. The effects of CD437 on *c-Fos* expression was not restricted to UMSCC22B cells but was observed in other HNSCC cell lines as well. Importantly, CD437 induced *c-Fos* expression in cell lines such as UMSCC14B, UMSCC17B, MDA886Ln, SqCC/Y1, and TR146 that underwent apoptosis but not in cell lines such as 183A and 1483 that failed to undergo apoptosis after exposure to CD437 (Fig. 3C). Therefore, *c-Fos* may be an important gene that attributes to CD437-induced apoptosis in HNSCC cells.

Suppression of Squamous Differentiation by CD437 and Other Retinoids. A comparison of the effects of CD437 and other retinoids on squamous differentiation in the UMSCC22B is shown in Fig. 4A. Because CD437 induces rapid apoptosis in various cells when used at 1 μM, we used a lower concentration (0.01 μM) to allow the cells to differentiate after several days treatment. All these retinoids, at 0.01 μM, decreased mRNA levels of the squamous differentiation markers *CK1* (Fuchs and Green, 1980), *involucrin* (Murphy et al., 1984; Eckert and Green, 1986), and *Spr1* (Tesfaigzi et al., 1993), indicating suppression of squamous differentiation. Interestingly, some retinoids such as Ch55, SR3985, AGN193273, AGN193078, and AGN190521, which did not induce apoptosis, were more potent in suppressing squamous differentiation than CD437 and CD2325, which induced apoptosis. Moreover, some of these retinoids were also more potent growth inhibitors at 0.01 μM than either CD437 or CD2325 (Fig. 4B).

Inhibition of CD437-Suppressed Squamous Differentiation by an RAR-Selective Antagonist. To determine whether RARs are involved in mediating suppression of squamous differentiation by the above-mentioned retinoids, we used the pan RAR-antagonist AGN193109 and found that it blocked the suppression of squamous differentiation markers *CK1*, *involucrin*, and *Spr1* by CD437, CD271, SR3985, and AGN190521 (Fig. 5A). Unexpectedly, the antagonist AGN193109 alone enhanced the expression of *CK1*, *Spr1*, and *involucrin* (Fig. 5A). Currently, we do not know why the AGN193109 stimulates the expression of these genes. It is possible that it antagonizes the effects of endogenous retinoids found in the serum used as growth medium supplement. Alternatively, this antagonist may possess an intrinsic pharmacological activity as an inverse agonist as demonstrated previously for the induction of differentiation marker MRP-8 in differentiating ECE16-1 cervical cells (Thacher et al., 1999). Another possibility is that the effect of the antagonist is mediated by a receptor-independent mechanism. To address this issue, we examined the effects of the pan RAR agonist ATRA and the pan RAR antagonist AGN193109 alone and in combination at different molar ratios on CK1 expression in UMSCC22B cells, expecting that if they both act via the RARs then they should interfere with each other's

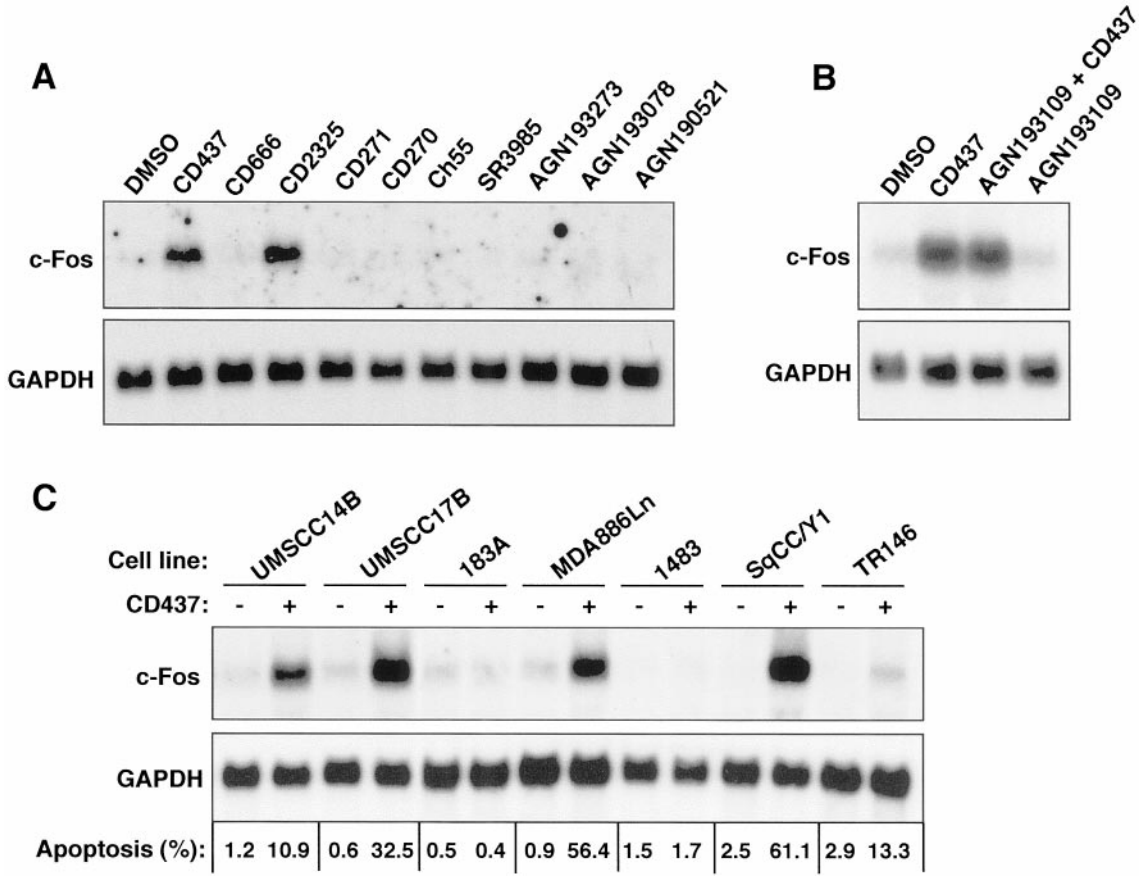


Fig. 3. Involvement of *c-Fos* in CD437-induced apoptosis in human HNSCC cells. A, differential modulation of CD437 and other retinoids on *c-Fos* expression in UMSCC22B cells. The cells were treated with 1 μM each of the indicated retinoids for 7 h. B, effect of AGN193109 on CD437-induced *c-Fos* expression in UMSCC22B cells. The cells were cotreated with 1 μM CD437 and 10 μM AGN193109 for 7 h. C, induction of *c-Fos* expression by CD437 in different HNSCC cell lines. The cells were treated with 1 μM CD437 for 7 h. Apoptosis was measured after a 24-h treatment by the TUNEL assay as described under *Materials and Methods*. For Northern analysis, 20 μg of total RNA was subjected to electrophoresis in an agarose gel and blotted to a nylon membrane. The procedures for total RNA purification and Northern blotting were described under *Materials and Methods*.

effects. Indeed, Fig. 5B shows that although AGN193109 alone and ATRA alone exerted opposite effects on *CK1* expression, in combination they appear to act as mutual antagonists, suggesting that the effect of AGN193109 is mediated by RARs. Therefore, we propose that the suppression of squamous differentiation by CD437 and other retinoids is mediated by RARs because their effect can be antagonized by AGN193109.

Discussion

CD437 was originally identified as an RARγ-selective retinoid (Bernard et al., 1992) and was more recently found to also transactivate RARβ (Dawson et al., 1998). Despite the receptor selectivity of this retinoid, several studies, including our own, have indicated that many of the effects of CD437 appear to be independent of the classical retinoid receptor-signaling pathway (Shao et al., 1995; Hsu et al., 1997; Sun et al., 1997a). In this investigation, we have demonstrated for the first time that CD437 can affect cellular phenotype by means of the nuclear retinoid receptors in human cancer cells. Because CD437 induces rapid apoptosis in various cells when used at 1 μM, we assumed that the cells die before they have an opportunity to differentiate. Therefore, to be able to observe the effects of CD437 on squamous differentiation, which may require several days of treatment, we have used a lower concentration (0.01 μM) than that used to induce apoptosis (1 μM).

More than 90% of human head and neck cancers, which include cancers of the larynx, pharynx, oral cavity, and tongue, are squamous cell carcinomas (Vokes et al., 1993). Some of these carcinomas exhibit characteristics of keratinizing squamous differentiation, which, however, is considered to be an abnormal differentiation when it occurs in the nonkeratinizing oral mucosa (Lotan, 1995). In this study, we used the human HNSCC cell line UMSCC22B, which we had previously found to be very sensitive to growth inhibition by various retinoids (Sun et al., 2000). Herein, we have shown that this cell line expresses several squamous differentiation markers (Fig. 4). Because natural and synthetic retinoids have been shown to suppress squamous cell differentiation in various SCC cell lines, including HNSCCs (Lotan, 1995), we examined the ability of CD437 and several other retinoids to do so in the UMSCC22B cells. We found that CD437, as well as several other retinoids, was indeed capable of suppressing the expression level of *Spr1*, *CK1*, and *involucrin*. The finding that the pan-RAR antagonist AGN193109 inhibited the suppressive effect of CD437 on squamous differentiation

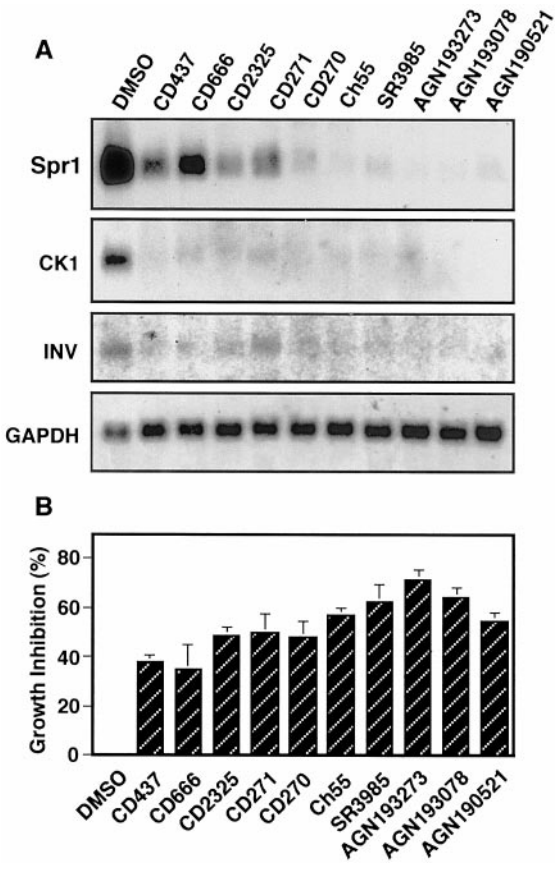


Fig. 4. Suppression of the expression of squamous differentiation markers (A) and growth inhibition (B) by CD437 and other retinoids in UMSCC22B cells. The cells were treated with 0.01 μM of the retinoids for 3 days (A) or 6 days (B). Twenty micrograms of total RNA was subjected to electrophoresis in an agarose gel and blotted to a nylon membrane. The procedures for total RNA purification and Northern blotting and growth inhibition were described under *Materials and Methods*. INV, involucrin.

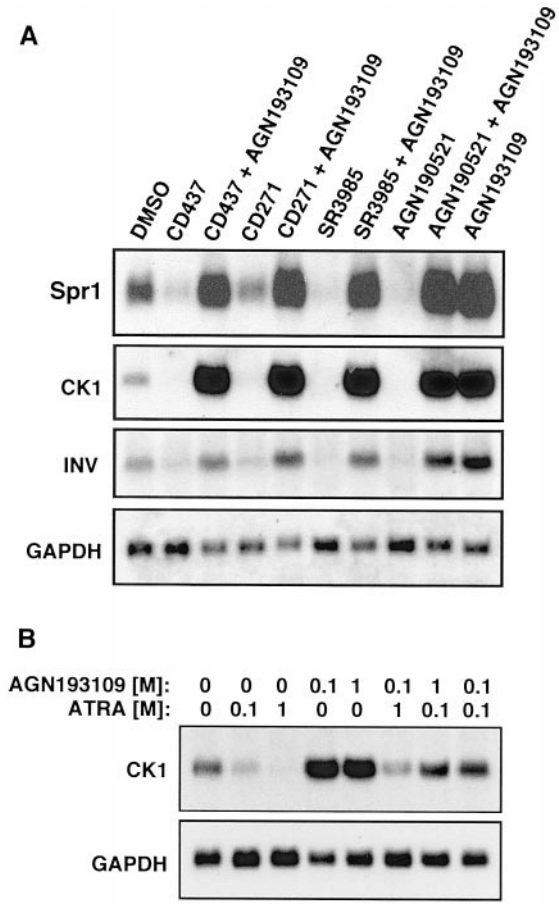


Fig. 5. Effect of RAR pan antagonist AGN193109 on suppression by CD437 of the expression of squamous differentiation markers (A) and effect of the pan RAR agonist ATRA on enhancement of *CK1* expression by AGN193109 (B) in UMSCC22B cells. A, cells were treated concurrently with 1 μM AGN193109 and 0.01 μM CD437 or other indicated retinoids for 3 days. B, cells were treated with the indicated concentrations of ATRA alone or AGN193109 alone or combination of ATRA and AGN193109 for 4 days. Twenty micrograms of total RNA was loaded per lane. The procedures for total RNA purification and Northern blotting were described under *Materials and Methods*. INV, involucrin.

marker expression indicates that this suppressive effect requires retinoid receptors. Northern blot analysis of total RNA from UMSCC22B cells indicated that these cells express RARα and RARγ but they do not express RARβ (S.-Y. Sun, P. Yue, and R. Lotan, unpublished data). Furthermore, RARβ could not be induced by ATRA (S.-Y. Sun, P. Yue, and R. Lotan, unpublished data). Because CD437 is an RARβ/γ-selective retinoid, it is plausible to assume that RARγ mediates the effect of CD437 in the UMSCC22B cells. Although effective, CD437 was less potent than several other retinoids examined for growth inhibition and for suppression of squamous differentiation at 0.01 μM (Fig. 4).

CD437 and the structurally related CD2325, which contains an adamantyl group (Table 1), were the only retinoids among the 10 examined that were able to induce apoptosis within 24 h at 1 μM concentration. However, most of these retinoids were able to inhibit growth after a 6-day treatment (Fig. 1). Thus, CD437 and CD2325 were distinct from other receptor-selective retinoids in their ability to induce apoptosis, suggesting that this effect may be independent of retinoid receptors. In contrast to the effect on differentiation, the induction of apoptosis by CD437 could not be inhibited by the pan-RAR antagonist AGN193109 (Fig. 2). This finding is similar to our previous observations in other cell lines (Sun et al., 1997a). Like the effect on apoptosis, the ability of CD437 to induce *c-fos* mRNA was also RAR independent. Our data also support an involvement of *c-Fos* in CD437-induced apoptosis because *c-Fos* expression was selectively up-regulated by CD437 and CD2325, but not by other retinoids that failed to induce apoptosis (Fig. 3A). Furthermore, our finding that sensitivity to CD437-induced apoptosis among several HNSCC cell lines correlated to increased *c-fos* mRNA expression also points to the possible role of *c-Fos* in mediating CD437-induced apoptosis. The involvement of activator protein-1 (c-Jun and c-Fos) (Schadendorf et al., 1996; Li et al., 1998) in CD437-induced apoptosis in other cancer cell types such as melanoma (Schadendorf et al., 1996) and lung carcinoma (Li et al., 1998) has been suggested. Furthermore, transfection of a dominant negative c-Jun, which can form a dimer with c-Fos and thereby interferes c-Fos function, was found to prevent apoptosis induction by CD437 in lung cancer cells (Li et al., 1998). Similar experiments may be required to determine conclusively whether *c-Fos* induction by CD437 in the UMSCC22B cells is important for the subsequent apoptosis.

Our previous studies demonstrated that CD437-induced apoptosis in human lung cancer cells could be mediated by p53-dependent and/or -independent mechanisms, depending on cell lines (Sun et al., 1999b,c). In this study, we found that the expression of p53 target genes such as *Killer/DR5* and *Bax* was not modulated by CD437 although this cell line has wild-type *p53* (Sun et al., 2000). Thus, it is unlikely that p53 plays a role in CD437-induced apoptosis in the UMSCC22B cells.

It appears that among the genes we examined in this study, CD437 induced only *c-Fos* expression in UMSCC22B cells. This effect is different from the findings in other types of cancer cell where CD437 increased the expression of *Killer/DR5*, *Bax*, *c-Myc*, and *c-Jun* (Li et al., 1998; Sun et al., 1999a,b,c; 2000). Therefore, we suggest that different apoptotic pathways may be involved in CD437-induced apoptosis in different cell lines.

This is the first report on modulation of differentiation of cancer cells by CD437. All previous studies have failed to observe any effect of CD437 on differentiation of cancer cells despite their responsiveness to ATRA. For example, CD437 did not induce differentiation of neuroblastoma (Meister et al., 1998), HL60 myeloid leukemia (Hsu et al., 1997), or NB4 human promyelocytic leukemia cells (Falanga et al., 1998). However, it has been reported that CD437, at 0.077 μM, can initiate glandular metaplasia of hair vibrissa follicles in embryonic mouse upper-lip skin explants (Blanchet et al., 1998). Insofar as induction of glandular metaplasia can be viewed as suppression of squamous differentiation, the effects of CD437 on HNSCC22B may be mechanistically similar to the effect of this retinoid on mouse skin.

In conclusion, we have shown that CD437 can act by at least two mechanisms in the UMSCC22B cells depending on the dose used: one mediated by a receptor-independent pathway (e.g., induction of apoptosis and induction of *c-Fos*) and the other by a receptor-dependent pathway (e.g., suppression of squamous cell differentiation).

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