Responsiveness to the Retinoic Acid Receptor-selective Retinoid LGD1550 Correlates with Abrogation of Transforming Growth Factor α/Epidermal Growth Factor Receptor Autocrine Signaling in Head and Neck Squamous Carcinoma Cells

Miriam Lango, Abbey L. Wentzel, John I. Song, Sichuan Xi, Daniel E. Johnson, William W. Lamph, Lori Miller, and Jennifer Rubin Grandis


ABSTRACT

Purpose: The use of retinoids to prevent and/or treat cancers, including head and neck squamous cell carcinoma (HNSCC), has been limited by the development of resistance and unwanted side effects. Retinoid-selective retinoids are potentially less toxic than nonselective compounds. The present investigation was undertaken to determine the mechanism of responsiveness to a retinoic acid receptor-selective retinoid (LGD1550) that has shown antitumor efficacy in a xenograft model of HNSCC.

Experimental Design: A series of HNSCC cell lines were characterized with respect to proliferation and apoptosis after LGD1550 treatment. Relative responsiveness to LGD1550 was examined with respect to modulation of epidermal growth factor receptor (EGFR) signaling pathways.

Results: Cells were either growth inhibited and underwent apoptosis or were resistant to treatment with this compound. Retinoids have been shown to decrease the gene transcription rates of transforming growth factor (TGF)-α and EGFR in HNSCC. LGD1550 responsiveness was accompanied by decreased expression of TGF-α, EGFR, and modulation of EGFR signaling pathways, including signal transducers and activators of transcriptions and mitogen-activated protein kinase. In contrast, EGFR autocrine signaling pathways were not altered in HNSCC cells that were resistant to the growth inhibitory effects of LGD1550.

Conclusions: These results suggest that there is a correlation between the efficacy of receptor-selective retinoids and modulation of TGF-α/EGFR signaling in HNSCC. Therefore, alterations of these signaling pathways may serve as a biomarker of clinical response.

INTRODUCTION

Retinoids, including vitamin A and its synthetic and non-synthetic derivatives, modulate fundamental cellular processes, including cell growth, differentiation, and apoptosis. Retinoid-dependent signaling pathways have been shown to play a role in suppression of carcinogenesis, thereby prompting the evaluation of these agents in preventative and therapeutic settings. Treatment with 13-cis-retinoic acid has been reported to delay the development of second primary tumors, a major cause of mortality in HNSCC patients who have received successful treatment of their primary tumor (1). 13-cis-retinoic acid has also been shown to cause clinical resolution of upper aerodigestive tract premalignant lesions (2, 3), although treatment appears to be less effective in genetically advanced lesions (4, 5). Resistance to retinoids can develop and has been associated with malignant progression. The mechanism(s) of this resistance remains incompletely understood.

Alternative synthetic compounds have been developed in the last several years in an effort to maximize efficacy and decrease the rate of therapy-related toxicity leading to noncompliance in up to one-third of patients. The rationale for the development of receptor-selective retinoids is based on the observation that specific downstream signaling pathways are preferentially involved in carcinoma prevention. Two types of nuclear receptors, the RARs, and RXRs, each with α, β, and γ subtypes, are thought to mediate the biological effects of retinoids by forming homo or heterodimers, binding to specific DNA sequences, and regulating the expression of target genes. RAR-selective retinoids are thought to preferentially activate signaling pathways involved in mitogenesis, compared with...
RXR-selective compounds that heterodimerize with a variety of other members of the steroid hormone receptor family (5, 6). In addition, some of the synthetic retinoids have been shown to promote apoptosis of tumor cells refractory to ATRA through retinoid receptor-independent mechanisms (7–12). This raises the possibility that some of the receptor-selective agents may be more efficacious at eliminating neoplastic or preneoplastic cells than the parental compounds.

The novel synthetic RAR-selective retinoid, LGD1550, binds all three RAR isoforms (α, β, and γ) with very low affinity for RXRs. A recent Phase I clinical trial demonstrated that this agent is generally well tolerated and, unlike other retinoids, does not induce its own metabolism (11). LGD1550 was shown previously to have antitumor efficacy in a head and neck cancer xenograft model, although the mechanism of these effects is incompletely understood (12). We have demonstrated previously that the antiproliferative effects of nonselective retinoids, including ATRA and 13-cis-retinoic acid, are mediated via abrogation of EGFR and TGF-α gene transcription, an early event in HNSCC tumorigenesis (13). In HNSCC cells, TGF-α/EGFR autocrine signaling appears to be mediated by constitutive activation of several STAT proteins, including Stat3 and Stat5 (14–18). We have shown previously that constitutive Stat3 activation is down-modulated by ATRA, 13-cis-retinoic acid, as well as by an RXR-selective retinoid (LGD1069) in HNSCC cells (19).

The present study was undertaken to examine the effects of LGD1550 on HNSCC cell proliferation and correlate retinoid-mediated growth inhibition with effects on TGF-α/EGFR autocrine signaling pathways. In the HNSCC cell lines that were growth inhibited by LGD1550 treatment, TGF-α and EGFR autocrine pathways were down-modulated. In contrast, HNSCC cells that were resistant to the growth inhibitory effects of LGD1550 did not demonstrate abrogation of autocrine signaling. LGD1550 was also found to induce apoptosis in responsive HNSCC cells. Elucidation of the mechanism of retinoid action in both retinoid-susceptible and resistant cells will facilitate the design of more effective therapeutic strategies. Monitoring of biomarkers can potentially be used to identify patients who are likely to respond to retinoid treatment.

MATERIALS AND METHODS

Reagents and Cell Lines. LGD1550 (2E.4E.6E)-7-(3,5-di-tert-butylphenyl)-3-methylocta-2,4,6-trienoic acid and LGD1069 (Targetrin) were generously provided by Dr. William Lamph (Ligand Pharmaceuticals, Inc., San Diego, CA). The compound was resuspended in DMSO, which was also used as a vehicle control. The HNSCC cell lines used in this study are part of a collection established in the Department of Otolaryngology at the University of Pittsburgh (PCI-37a, PCI-15b; Ref. 20). Several cell lines (UPCI: SCC141, UPCI: SCC52, UPCI: SCC203, UPCI: SCC32, UPCI: SCC66, and UPCI: SCC42) were kindly provided by Dr. Susanne M. Gollin (Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA). The 1483 cell line is a well-characterized cell line derived from a tumor of the oropharynx that grows well as xenografts in athymic nude mice as described (21). Cell lines were maintained in DMEM (Cellgro, Washington, DC) supplemented with 12% heat inactivated FCS (Invitrogen), plus 1% penicillin/streptomycin mix (Invitrogen, Carlsbad, CA) at 37°C with 5% CO2.

Cellular Proliferation Assays. Proliferation rates of HNSCC cell lines were measured by cell count experiments using vital dye (erythrosin B) exclusion. Results were confirmed using an MTT assay. Cells were treated with LGD1550 at a concentration of 1 × 10^-7 M or 1 × 10^-9 M. Control cells were treated with an equal volume of DMSO. Cells were plated and treated in duplicate in cell count experiments and in triplicate in MTT proliferation studies. Proliferation assays were repeated at least two times in separate experiments. Statistical analysis (unpaired t test) was performed using GraphPad Prism Software (GraphPad Software, San Diego, CA).

Northern Blot Analysis. Cells were plated, allowed to reach 80–90% confluence, and then treated with LGD1550 1 × 10^-8 M for 24 h. RNA was harvested using the Qiagen RNA Easy Kit according to the instructions of the manufacturer. Twenty micrograms of total RNA were loaded on an agarose-formaldehyde gel, blotted onto Zetabind (AMF-Cuno, Meriden, CT), and bound to the membrane by brief exposure to UV irradiation. The membranes were probed with TGF-α, EGFR, and 18S DNA probes, as described previously (22). Nucleic acid probes were radiolabeled using [32P]dCTP. After hybridization, membranes were washed and exposed to Kodak XAE film with an intensifying screen at -80°C for 1–7 days. TGF-α and EGFR levels were quantified relative to 18S RNA bands using two-dimensional densitometry.

Immunoblotting. Cells were treated with LGD1550 or LGD1069 at a concentration of 1 × 10^-8 M for 48 h. Cells were lysed in detergent containing 1% NP40, 0.1 mM phenylmethylsulfonyl fluoride, 1 mg/ml leupeptin, and 1 mg/ml aprotinin. Protein levels were determined using the Bio-Rad Protein Assay method (Bio-Rad Laboratories, Hercules, CA). Fifty micrograms of total protein were separated on a 10% SDS-PAGE gel and transferred to nitrocellulose membranes (MSI, Westboro, MA). Prestained molecular weight markers (Life Technologies, Inc., Gaithersburg, MD) were included in each gel. Membranes were blocked using 5% BSA/Tris-buffered saline with Tween 20 solution for 1 h, rinsed with Tris-buffered saline with Tween 20, then incubated for 90 min with a mouse antihuman EGFR monoclonal antibody (Transduction Labs, Lexington, KY). Membranes were incubated with a horseradish peroxidase-conjugated secondary antibody (Bio-Rad Laboratories). EGFR protein signal was detected using Luminol Reagent (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Membranes were exposed to Kodak X OMAT film for 5–15 s. For the signal transduction studies, cells were plated and treated with LGD1550 1 × 10^-7 M in DMEM supplemented with 10% FBS. Control cells were treated with DMSO. Cell lysates were harvested at 2, 4, 8, 24, and 48 h. Antibodies included p42/44 MAPK, phospho-MAPK (Thr202/Tyr204), and phospho-AKT (Ser472). All were purchased from Cell Signaling Technology (Beverly, MA). A β-actin antibody was used as a loading control was purchased from Oncogene Products (Boston, MA).

ELISA. When cells were 80–90% confluent, they were treated with LGD1550 (1 × 10^-7 M) for 48 h. Cell lysates were collected. Protein was extracted and quantified. Protein extracts were tested for TGF-α protein expression using a commercially...
available ELISA assay (Oncogene Research Products) according to the manufacturer's instructions.

Flow Cytometry. Cells were plated in six-well plates and treated with LGD1550 (1 × 10^{-7} M) or DMSO in DMEM supplemented with 1% FBS when ~40–50% confluent. Supplemental media with LGD1550 or DMSO were added to wells on alternate days. Cells were treated for 1, 3, or 5 days, and analyzed by flow cytometry using PI. In a separate experiment, cells were treated with LGD1550 (1 × 10^{-7} M) versus DMSO in DMEM when 70% confluent. Cells were treated for 1, 12, or 24 h in triplicate, and both attached and detached cells were harvested, pooled, and immediately analyzed by 2-dye flow cytometry using YoPro/PI (23).

EMSAs. Whole cell extracts were prepared, and EMSAs were performed on 4% native polyacrylamide gels as described (24). Stat3 activation was assessed by determining the presence of DNA-binding activity as manifested by SIF activity, where SIF-A represents Stat3 homodimers, SIF-B represents Stat1/3 heterodimers, and SIF-C represents Stat1 homodimers. For supershift experiments, extracts were preincubated with a Stat3α polyclonal antibody (C-20; Santa Cruz Biotechnology). Quantitation of the Stat3 signal was performed by scanning the SIF-A band using a Molecular Dynamics Personal Densitometer SI and IMAGEQUANT software. For Stat5 EMSA, complementary duplex oligonucleotides were synthesized based on the published sequences of STAT DNA-binding elements with the addition of GGGG at the 5' termini to allow radiolabeling as described previously (β-casein promoter: 5’-AGATTTCTAG-GAATCAAATC-3'; Ref. 18). For supershift experiments, extracts were preincubated with Stat5α polyclonal antibody (L-20; Santa Cruz Biotechnology) or Stat5b polyclonal antibody (N-20; Santa Cruz Biotechnology). Stat4 EMSAs were performed using Stat4 Gel Shift Oligonucleotides (Santa Cruz Biotechnology).

Table 1 Summary of the effects of LGD1550 on HNSCC proliferation

<table>
<thead>
<tr>
<th>HNSCC cell line</th>
<th>% growth inhibition at day 6</th>
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<tbody>
<tr>
<td>UPCI: SCC 52</td>
<td>81</td>
</tr>
<tr>
<td>PCI-37a</td>
<td>68</td>
</tr>
<tr>
<td>PCI-15b</td>
<td>62</td>
</tr>
<tr>
<td>UPCI: SCC 141</td>
<td>45</td>
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<tr>
<td>UPCI: SCC 142</td>
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<td>13</td>
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<tr>
<td>UPCI: SCC 32</td>
<td>3</td>
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Fig. 1 Characterization of HNSCC cell susceptibility to LGD1550-mediated growth inhibition. Representative HNSCC cell lines were treated with 1 × 10^{-7} M LGD1550 (Δ) or DMSO control (□) on days 0 and 3. Proliferation rates of HNSCC cell lines were measured by cell count experiments using vital dye (erythrosin B) exclusion. Control cells were treated with an equal volume of DMSO. Proliferation assays were repeated at least two times in separate experiments. Error bars show SE. PCI-37a (A) and UPCI: SCC52 (B) are two representative cell lines growth inhibited by LGD1550. UPCI: SCC32 (C) and UPCI: SCC66 (D) were resistant to the antiproliferative effects of LGD1550.
RESULTS

HNSCC Cell Lines Demonstrate Differential Susceptibility to LGD1550. To determine the growth effects of LGD1550 in HNSCC, a series of HNSCC cell lines were screened for growth inhibition induced by this novel RAR-selective retinoid. HNSCC cells were variably susceptible to the growth inhibitory effects of LGD1550. HNSCC cell lines that were responsive to LGD1550 demonstrated a median growth inhibition of 54% by day 6. The growth response of representative cell lines is shown in Fig. 1 and Table 1. Retinoid-susceptible cell lines demonstrated dose dependent growth inhibition (data not shown). Response to treatment was highly reproducible using both cell counting and MTT assays.

LGD1550 Inhibits Growth in Susceptible HNSCC Cells by Inducing Apoptosis. The mechanism of LGD1550-mediated growth inhibition is not well understood. Synthetic retinoid compounds have been shown to induce apoptosis (8–12, 25). To evaluate the mechanism of LGD1550-mediated growth inhibition, a LGD1550-susceptible cell line (PCI-37a) was treated with LGD1550 or DMSO vehicle control for 24, 48, 72, or 96 h, in triplicate. Control cells were treated with an equivalent volume of DMSO at each time point, in triplicate. At the conclusion of the experiment, cells on the plates and in the media were harvested and pooled, and immediately analyzed by 2-dye flow cytometry using YoPro/PI. By 24 h, a 2-fold increased induction of apoptosis in LGD1550-treated cells compared with vehicle control was demonstrated (B4 quadrant). ■, control; □, LGD1550.

Fig. 2 The growth inhibitory effect of LGD1550 in susceptible HNSCC cell lines is accompanied by increased apoptosis. Flow cytometry was performed in an LGD1550 responsive cell line (PCI-37a). LGD1550 causes decreased viability (A) and increased necrosis (B) at each time point tested. In a separate experiment (C and D), PCI-37a cells were treated with LGD1550 (1 × 10^{-7} M) or DMSO in DMEM for 1, 12, or 24 h in triplicate, and both attached and detached cells were harvested, pooled, and immediately analyzed by 2-dye flow cytometry using YoPro/PI. By 24 h, a 2-fold increased induction of apoptosis in LGD1550-treated cells compared with vehicle control was demonstrated (B4 quadrant). ■, control; □, LGD1550.

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rate of apoptosis in LGD1550-treated cells increased over time with a doubling of the rate of apoptosis by 24 h (Fig. 2).

**LGD1550 Down-Modulates TGF-α and EGFR Expression in LGD1550-susceptible Cell Lines.** We have shown previously that nonselective retinoids, including 13-cis-retinoic acid and ATRA, may mediate growth effects by modulating TGF-α/EGFR autocrine signaling (19). In the present study, HNSCC cell lines were tested for modulation of TGF-α/EGFR expression using Northern blotting, Western blotting, and ELISA. TGF-α and EGFR mRNA levels were found to be down-modulated in LGD1550 susceptible cells but not in LGD1550 resistant cells (Figs. 3A and 4A). Similarly, EGFR protein expression was found to be down-modulated by a mean of 66% in LGD1550 susceptible cells using Western blotting, whereas LGD1550 did not significantly modulate EGFR protein expression in retinoid resistant cells (Fig. 3B). Determination of TGF-α protein levels using an ELISA assay revealed down-modulation of TGF-α protein expression in response to treatment with LGD1550 in cells that were growth inhibited by this compound but not in resistant cells (Fig. 4B).

**Modulation of Activation of STATs 3 and 5 Is Correlated with Growth Inhibition by LGD1550.** We have shown previously that in HNSCC, TGF-α/EGFR signaling is linked to constitutive Stat3 activation in vitro and in vivo (15, 16). Constitutive Stat3 activation was decreased in response to treatment with nonselective and RXR-selective retinoids (26). In the present study, treatment with LGD1550 resulted in a decrease in Stat3 activation in LGD1550-susceptible cells but not in LGD1550-resistant cells (Fig. 5A). The presence of Stat3 in the gel shift complex was confirmed by supershift analysis using specific antisera (Fig. 5B). These results were also corroborated using Western blotting with a phosphotyrosine-specific antibody to Stat3 (data not shown). In addition to Stat3, we have also reported that activation of Stat5 is downstream of EGFR in HNSCC cells (18). Interestingly, Stat3 activation was increased in response to LGD1550 in the resistant cell lines, suggesting a potential association between Stat3 activation and retinoid resistance. To determine whether Stat5 activation was modulated...
by LGD1550 in responsive cells, PCI-37a was treated with LGD1550 followed by EMSA. As shown in Fig. 5C, constitutive activation of Stat5 was decreased by LGD1550 in this sensitive cell line. To demonstrate the specificity of LGD1550-mediated modulation of STATs 3 and 5, we also examined the effects of this selective retinoid on Stat4 activation. As shown in Fig. 5D, there was no modulation of Stat4 by LGD1550 in LGD1550-responsive HNSCC cells.

**LGD1550 Down-Modulates MAPK but not AKT Activation in LGD1550-susceptible HNSCC Cells.** MAPK activation has been implicated in EGFR-mediated signal transduction in HNSCC cells (27). We therefore examined the effects of LGD1550 on MAPK in a susceptible HNSCC cell line (PCI-37a). As shown in Fig. 6, LGD1550 caused a sustained down-modulation of phosphoMAPK that was noted at 4 h and persisted for 24 h. EGFR stimulation is known to activate phosphotyrosine/YAkt-3 kinase and AKT as well as MAPK pathways in HNSCC cells (28–30). However, alterations in the levels of activated AKT were observed in LGD1550-responsive, as well as in resistant, HNSCC cell lines, suggesting that activation of AKT is not involved in LGD1550 resistance (Fig. 6D). We reported previously that the RXR-selective compound, LGD1069/Targretin, abrogated STAT activation in HNSCC cell lines (19). Similar to the effects seen with LGD1550, MAPK activation was down-regulated in a LGD1069-responsive cell line after treatment with this RXR-selective retinoid (Fig. 6C).

**DISCUSSION**

Retinoid-induced modulation of cell growth, differentiation, and apoptosis is thought to be mediated primarily by nuclear retinoid receptors. Receptor-selective ligands have been developed in an effort to maximize antitumor efficacy and decrease therapy-related toxicity. It is unclear whether ligands

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**Fig. 5** Modulation of Stat3 activation is correlated with growth response to LGD1550. Whole cell extracts were prepared, and EMSAs were performed on 4% native polyacrylamide gels. Stat3 activation was assessed by determining the presence of DNA-binding activity as manifested by SIF activity, where SIF-A represents Stat3 homodimers. For supershift experiments, extracts were preincubated with a Stat3a polyclonal antibody as described in “Results.” In A, LGD1550 treatment (1 × 10−7 M for 2 h) abrogates constitutive Stat3 activation in SCCHN cell lines (PCI-37a and UPCI:SCC52) that are growth inhibited by LGD1550. The level of Stat 3 activation is actually increased in selected SCCHN cell lines (UPCI:SCC32 and UPCI:SCC66) that are resistant to the growth inhibitory effects of LGD1550. In B, the presence of Stat 3 in the gel shift complex is verified by supershift analysis of an LGD1550-susceptible cell line PCI-37a. C, down-modulation of Stat5 activation in a LGD1550-responsive cell line (PCI-37a) as shown by EMSA using a Stat5 response element from the β-casein promoter with supershift analysis to demonstrate that the band represents Stat5 DNA binding. D, lack of modulation of Stat4 activation by LGD1550 in a responsive HNSCC cell line (PCI-37a) with supershift analysis to demonstrate that the band represents Stat4 DNA binding.
that interact selectively with RAR or RXR, or with specific subtypes within these families, are more efficacious. In addition, several of the novel synthetic retinoids are known to mediate receptor-independent apoptosis (8–12, 25). In the present study, we evaluated the mechanism and growth effects of an RAR-selective retinoid, LGD1550, on a panel of HNSCC cell lines. This agent has been evaluated in a Phase I clinical setting with little toxicity reported (11). Although LGD1550 has been shown to demonstrate antitumor efficacy in a xenograft model of HNSCC, mechanistic studies were not reported. We found that LGD1550 caused dose-dependent growth inhibition of a majority of the HNSCC cell lines tested. Retinoid responsiveness in HNSCC cells corresponded with modulation of selected TGF-α/EGFR autocrine signaling pathways. Specifically, EGFR down-modulation was linked with decreased activation of Stat3, Stat5, and MAPK but not AKT activation. Determination of the signaling pathways involved in retinoid responsiveness will facilitate the design of therapeutic strategies that can overcome resistance.

The heterogeneity of retinoid susceptibility observed in HNSCC cells in vitro mimics clinical patterns of retinoid resistance. Neoplastic or preneoplastic lesions result from an outgrowth of cells with clonal genetic alterations, some of which may confer resistance to retinoids, e.g., patients with genetically advanced premalignant lesions (e.g., loss of heterozygosity, chromosomal polysomy, and p53 status) do not respond to treatment, and their lesions often progress to invasive cancer (4, 31). The molecular basis of retinoid resistance is incompletely understood but is thought to involve dysfunction at the level of the retinoid receptors. Novel synthetic agents have been developed which were subsequently shown to have activity against malignant cells resistant to ATRA. In the present study, we

**Fig. 6** LGD1550 causes down-modulation of MAPK, but not AKT, activation in susceptible but not resistant HNSCC cells. Sensitive (PCI-37a) and resistant (UPCI:SCC32) cells were plated and treated with LGD1550 (1 × 10^{-7} M) in DMEM supplemented with 10% FBS. Control cells were treated with DMSO. Cell lysates were harvested at 2, 4, 6, and 24 h. A, representative immunoblotting demonstrating that LGD1550 treatment resulted in down-modulation of phosphorylated MAPK and phosphoserine AKT in the absence of alterations in levels of total MAPK protein. In contrast, levels of phosphoserine AKT were similarly modulated in LGD1550 responsive and resistant cell lines. β-actin expression is shown as a control for loading. B, densitometric analysis of cumulative immunoblotting results. C, immunoblotting of pMAPK after treatment with the RXR-selective retinoid, LGD1069 (1 × 10^{-7} M for 24 h), in a LGD1069-responsive cell line (PCI-37a).
found that retinoid responsiveness in HNSCC cells corresponds with modulation of TGF-α/EGFR autocrine signaling in vitro. We have shown previously that the nonselective retinoid ATRA regulates TGF-α/EGFR gene transcription rates (13). The EGFR gene contains a retinoid response element in its promoter and has been shown to be regulated by retinoid receptors (32). We have found that both the RXR-selective retinoid LGD1550 (Targetit; Ref. 19) and the RAR-selective retinoid LGD1550 are able to modulate TGF-α/EGFR autocrine signaling, suggesting that RAR-RXR heterodimers may be responsible for mediating these effects. Others have reported decreased EGFR expression in HNSCC cell lines or patients treated with retinoic acid (33, 34).

Several pathways can be activated by EGFR stimulation, including MAPK, AKT, and STATs. RAR-selective retinoids have been shown to suppress EGFR-mediated MAPK activation, causing growth inhibition in the absence of AKT, p38, and c-Jun NH₂-terminal kinase activation in a cervical carcinoma cell line (14). We reported previously that constitutive activation of Stat3 and Stat5 is linked to TGF-α/EGFR signaling and is critical for HNSCC proliferation (15, 18). The antiproliferative effects of nonselective and RXR-selective retinoids in HNSCC have been associated with abrogation of constitutive Stat3 activation (19). In the present study, we examined the correlation between retinoid responsiveness and modulation of the TGF-α/EGFR autocrine pathway. Down-modulation of TGF-α/EGFR autocrine signaling in LGD1550-resistant cells was associated with a decrease in constitutive Stat3 and Stat5 activation. This response was not elicited in HNSCC cells that were resistant to the growth inhibitory effects of LGD1550, suggesting that retinoid-induced growth inhibition is mediated through down-modulation of these pathways. Decreased MAPK activation was also demonstrated in LGD1550-resistant cells, indicating that LGD1550 effects may be routed through several pathways which cooperate to generate uncontrolled growth. Both MAPK and Stat3 activation have been implicated in induction of cyclin D1, cdk4/6 activation, and G1 progression (14, 35), as well as inhibition of apoptosis through up-regulation of the antiapoptotic protein, Bcl-xL (16, 36). However, MAPK, but not Stat3 activation, by endothelial growth factor is necessary for survival of normal and immortalized human keratinocytes (36). In these normal cells, EGFR-dependent MAPK activity contributes to Bcl-xL expression and survival. In contrast, A431 vulvar SCC and HNSCC cells are characterized by constitutively phosphorylated Stat3, which may be further stimulated by exogenous epidermal growth factor, implicating Stat3 signaling in carcinoma-specific signaling pathways. We have shown previously that constitutive activation of Stat3 signaling abrogates apoptosis in HNSCC (16). In the present study, induction of apoptosis in an LGD1550-resistant HNSCC cell line contributes to decreased proliferation.

The results suggest that an RAR-selective retinoid LGD1550 may be efficacious in the prevention and/or treatment of head and neck cancer. LGD1550 inhibits HNSCC cell proliferation by selectively down-modulating TGF-α/EGFR autocrine signaling pathways, including Stat3 and MAPK in susceptible cell lines. It is possible that the combination of retinoids and EGFR antagonists may prove more efficacious than either therapy alone. By determining the downstream effects of retinoid susceptibility, it may be possible to develop strategies to overcome retinoid resistance and monitor modulation of biomarkers to identify patients who will respond to treatment with receptor-selective retinoids.

REFERENCES


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