Loss of c-myc Repression Coincides with Ovarian Cancer Resistance to Transforming Growth Factor β Growth Arrest Independent of Transforming Growth Factor β/Smad Signaling

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ABSTRACT

Many epithelial carcinomas, including ovarian, are refractory to the antiproliferative effects of transforming growth factor (TGF) β. In some cancers, TGF-β resistance has been linked to TGF-β receptor II (TβR-II) and Smad4 mutations; however, in ovarian cancer, the mechanism of resistance remains unclear. Primary ovarian epithelial cell cultures were used as a model system to determine the mechanisms of TGF-β resistance. To simulate in vivo responses to TGF-β, primary cultures derived from normal human ovarian surface epithelium (HOSE) and from ovarian carcinomas (CSOC) were grown on collagen I gel, the predominant matrix molecule in the ovarian tumor milieu. When treated with 5 ng/ml TGF-β for 72 h, HOSE (n = 11) proliferation was inhibited by 20 ± 21% on average. In contrast, CSOC (n = 10) proliferation was stimulated 5 ± 10% in response to TGF-β (a statistically significant difference in response when compared with HOSE; P = 0.001). To dissect the TGF-β/Smad signaling pathway we used a quantitative RNase protection assay (RPA) for measuring mRNA levels of TGF-β pathway components in 20 HOSE and 20 CSOC cultures. Basal mRNA levels of TGF-β receptors I and II, downstream signaling components Smad2, 3, 4, 6, 7, and the transcriptional corepressors Ski and SnoN did not show a statistically significant difference between HOSE and CSOC, and cannot explain their differential susceptibility to TGF-β-induced cell cycle arrest. To assess functional differences of the TGF-β pathway in TGF-β-sensitive HOSE and TGF-β-resistant CSOC, we measured Smad2/4 and 3/4 complex induction after TGF-β treatment. HOSE and CSOC showed equivalent Smad2/4 and 3/4 complex induction after TGF-β exposure for 0, 0.5, 2, and 4 h. It has been proposed that SnoN and Ski are corepressors of the TGF-β/Smad pathway and undergo TGF-β-induced degradation followed by reinduction of SnoN mRNA. However, our data show equivalent SnoN degradation in HOSE and CSOC, and equivalent SnoN mRNA induction after TGF-β treatment. Surprising, TGF-β-induced Ski degradation was not observed in HOSE or CSOC, suggesting that Ski may not function as a TGF-β/Smad corepressor in ovarian epithelial cells. These data implied that the TGF-β/Smad pathway remains functional in CSOC, although CSOC cells are resistant to antiangiogenic TGF-β effects. CSOC resistance to TGF-β coincided with the loss of c-myc down-regulation. These data suggest that TGF-β/Smad signaling is blocked downstream of Smad complex formation or that an alternate signaling pathway other than TGF-β/Smad may transmit TGF-β-induced cell cycle arrest in the ovarian epithelium.

INTRODUCTION

Ovarian cancer continues to be the most lethal gynecologic malignancy, accounting for >14,000 deaths per year (1). In the majority of patients, ovarian cancer is not detected until late stages. Consequently, little is understood about the molecular events causing the malignant transformation of normal ovarian epithelial cells or about the molecular processes regulating disease progression. We demonstrated previously that one of the molecular features of the malignant ovarian epithelium is an acquired resistance to the antiproliferative effects of TGF-β (2). Insensitivity to TGF-β-mediated growth arrest has been observed in many other epithelial carcinomas (3, 4). In gastric, colon, and pancreatic cancers, the loss of TGF-β growth inhibition has been attributed to mutations in TβR-II (5, 6), and mutations in the signaling components, Smad 2 and 4 (7, 8). However, in the case of ovarian cancer, TGF-β receptor and Smad mutations are infrequent events (9–12).

In humans, the TGF-β family is represented by three ligands, TGF-β1, TGF-β2, and TGF-β3, which exert their activity through a family of transmembrane serine/threonine kinase receptors, TβR-I and TβR-II (13, 14). A third receptor, TβR-III, does not contain a known signaling domain but can actively present ligand to TβR-II (15) and is believed to serve as a ligand repository. Ligand binding to TβR-II initiates a heteromeric complex formation between TβR-II and TβR-I, followed by phosphorylation of TβR-I by TβR-II. Activated TβR-I then phosphorylates and activates downstream signaling components, including Smads 2 and 3 (13). After being phosphorylated, Smad 2 and 3 form heteromeric complexes with Smad 4 followed by migration from the cytoplasm into the nucleus where these complexes bind to specific promoter elements either alone or in association with a DNA-binding subunit, and regulate TGF-β-mediated expression and/or repression of target genes (16). It is thought that Smad 6 and 7 inhibit TGF-β-mediated signaling by interfering with the phosphorylation of Smad 2 and 3 by TβR-I (13).

Smads promote transcriptional activation, at least in part through the recruitment of the general coactivators p300/cAMP response element-binding protein–binding protein, both large proteins with histone acetyl transerase activity (17). Smad complexes have also been shown to interact with transcriptional corepressors, including T cell growth inhibitory factor, and the proto-oncogenes Ski and SnoN, which modulate Smad-dependent transcription by recruiting histone deacetylases (17). Ski and SnoN are found to be associated with Smad 3 in the basal, unstimulated state. Upon TGF-β stimulation, this association dissolves, and Ski and SnoN undergo rapid degradation allowing signal transduction to proceed. To prevent perpetual ligand-dependent TGF-β signaling, TGF-β subsequently induces SnoN expression providing a negative feedback loop controlling Smad-dependent gene activation (16).

In most untransformed cell types TGF-β causes the down-regulation of c-Myc, a downstream target of the Smad pathway (18, 19). Because c-Myc has a short half-life, TGF-β-dependent down-regulation of c-Myc mRNA results in a rapid protein loss (18, 20) and appears to be required for growth inhibition (18, 21). c-Myc acts as a transcriptional activator of genes required to promote the G1–S phase
transition, or depending on the gene target, can also act as a transcriptional repressor (22). c-Myc has been shown to inhibit expression of p15^{ink4a}, a cyclin-dependent kinase 4 and 6 inhibitor (23). Therefore, one of the mechanisms in which c-Myc down-regulation by TGF-β results in cell growth inhibition seems to be the removal of c-Myc-mediated repression of p15^{ink4a} (18). Overexpression of c-Myc inhibits TGF-β antiangiogenic effects and additionally supports the conclusion that c-Myc down-regulation is one of the key events in the TGF-β growth-inhibitory response (18, 24).

In previous studies we demonstrated that the preliminary steps of the TGF-β/Smad signaling cascade remain intact and functional in ovarian adenocarcinomas (2). TβR-I and TβR-II mRNA are expressed in primary ovarian tumors, HOSE and CSOC, and TGF-β treatment induced TβR-I phosphorylation in HOSE and CSOC (2, 25). To additionally define the TGF-β tumor suppressor pathway in ovarian tumorigenesis we have examined the expression and functional status of downstream signaling components of the TGF-β/Smad pathway in HOSE and CSOC primary cultures. The significant role of ECM in cell signaling and phenotype determination has been well documented (26). To better recapitulate the in vivo ovarian tumor environment, we grew the primary cultures on collagen type I, the prevalent ECM molecule in the ovarian microenvironment to which CSOC cells show preferential adhesion (27). Using this model system, we evaluated the expression and functional status of the TGF-β receptors, Smad signal transducers, transcriptional corepressors and proto-oncogenes Ski and SnoN, and the downstream target c-myc. This is the first report to date analyzing Smad, Ski and SnoN basal expression, and TGF-β-dependent Smad complex formation in primary ovarian cultures. It is also the first report addressing TGF-β-induced degradation of Ski and SnoN in primary cultures, a model system more in vivo-like than the immortalized, transfected cell lines (28).

Our data show that acquired resistance of primary ovarian cancer cell cultures to TGF-β-mediated growth inhibition coincides with the loss of c-myc repression. Resistance appears to be independent of Smad expression or complex formation, because malignant cultures did not show altered Smad expression levels or altered Smad hetero- and homodimeric complex formation in response to TGF-β treatment. In addition, TGF-β resistance does not appear to be linked to altered regulation of the transcriptional corepressors SnoN and Ski. No change was observed in TGF-β-induced mRNA expression or protein degradation of Ski and SnoN in CSOCs compared with HOSEs.

**MATERIALS AND METHODS**

Primary Cell Cultures and Collagen I in Vitro Culture System. Primary cell cultures of normal HOSE and CSOC were established from surgical specimens as described previously (2, 29) under a protocol approved by the Cedars-Sinai Medical Center Institutional Review Board. HOSEs were derived from patients undergoing oophorectomy for benign conditions and CSOCs were derived from patients undergoing surgical cytoreduction for ovarian cancer. A total of 20 HOSE and 20 CSOC cultures derived from distinct patients were used in this study.

Collagen type I gels (Becton Dickinson Labware, Bedford, MA) were prepared at 3 mg/ml, which was determined to be the optimal concentration for cells to be plated in triplicate wells in 96-well plates on collagen I gels. Cells were allowed to attach overnight, followed by a 16 h serum starvation interval, and then treatment with 5 ng/ml TGF-β1 (Collaborative Biomedical Products) for 72 h. Control cells were incubated under identical conditions without TGF-β1. Three independent treatment assays were performed. Proliferation of TGF-β-treated cells was expressed as percentage of control cell proliferation.

RPA. Total RNA was isolated from 20 HOSE and 20 CSOC cultures grown on collagen I gels using TRI Reagent (MRC, Cincinnati, OH). A quantitative analysis of TGF-β pathway component mRNA expression levels (TGF-β ligands and receptors, Smads, and Ski and SnoN) was performed using RPA. For each determination, three independent evaluations were performed. A centrifugation step at 10,000 rpm for 10 min at 4°C was added immediately after TRI Reagent addition, to remove any residual collagen I gel. RPA was performed using the RPA III kit (Ambion, Austin, TX). In brief, 2 μg of total RNA per sample was hybridized with a radioactively labeled probe overnight at 42°C followed by digestion with 1:100 RNase A/RNase T1 mix at 37°C for 30 min to degrade unhybridized probe. The hybridized fragments were separated on a denaturing 5% polyacrylamide gel and visualized by autoradiography. Band intensity was determined by densitometric analysis of digitized gel images using One-D Scan analysis software (Scanalytics, Billerica, MA). Expression levels of G3PDH were used for normalization of mRNA sampling.

Preparation of RPA Probes. The template for the TβR-I probe was constructed as described (30, 31). Templates for TβR-II, Ski, SnoN, and G3PDH probes were prepared by PCR amplification using the following primers: Ski forward, AGA AAT TCG ACT ATG GCA ACA AGT A and Ski reverse, TCA AAG CCT TTT TAG GAG TAG AAG C; SnoN forward, ATG GCA CCT TTA TCT TCA TGT G and SnoN reverse, GCT GGG GTG TAA AAA TGA ATG T. PCR products were cloned into pCR II TOPO vector for in vitro transcription. The constructs were verified by DNA sequencing at the Cedars-Sinai DNA Sequencing Core Facility. The size of the RPA probes for TβR-I and II were designed to allow for simultaneous multiplex analysis of samples (377 and 476 bp, respectively). Antisense probes for the quantitation of Smad mRNA expression levels were synthesized using the hSma-Multi-Probe Template Set (PharMingen, San Diego, CA). This set contains templates for human Smad1, 2, 3, 4, 5, 6, 7, and 8, as well as for two housekeeping genes, L32 and G3PDH. All of the antisense RNA probes were synthesized by in vitro transcription using Riboprobe Combination Systems (Promega) with [α-32P]CTP. After transcription, the Smad multiprobe set was purified by ammonium acetate and ethanol precipitation according to the manufacturer’s specification. TβR-I, II, Ski, SnoN, and G3PDH probes were purified by PAGE in vitro transcription, and eluted from gel fragments by overnight incubation in Elution Buffer (Ambion) at 37°C.

Smad 2/4 and 3/4 complex formation was detected by immunoprecipitation with Western blot analysis. For HOSE and 5 CSOC cultures grown on collagen I gels were treated with TGF-β1 for 0.5, 2, and 5 h, followed by isolation of total cellular protein. Three independent TGF-β treatments were performed. Protein lysates were prepared using 1× radioimmunoprecipitation assay buffer [PBS, 1% NP0, 0.5% sodium deoxycholate, 0.1% SDS, leupeptin (0.5 μg/ml), phenylmethylsulfonyl fluoride (10 μg/ml), and sodium orthovanadate (0.18 mg/ml)], Protein concentrations were determined using the BCA colorimetric protein assay method (Pierce, Rockford, IL).

For quantitative assessment of Smad complex formation, an equal amount of total cellular protein (100 μg/sample) was immunoprecipitated using Smad4 antibody (1 μg/sample) and Protein A-Agarose conjugate (Santa Cruz Bio-technology, Inc.) overnight at 4°C. The samples were then separated on reducing 10% SDS-polyacrylamide gels, electrotransferred onto Immobilon-P membranes (Millipore Corp., Bedford, MA), and probed with Smad2 (S-20) and Smad3 (D-20) antibodies (1:100 dilutions) to detect the presence of Smad2/4 and Smad3/4 complexes. The complexes were detected using Vectastain ABC Elite kit (Vector Laboratories, Burlingame, CA) with horseradish peroxidase complex and diaminobenzidine as a chromogenic substrate. Ski, SnoN, and c-Myc protein expression by Western blot using 20 μg of total cellular protein per sample. Western blots were incubated with anti-Ski (A-20), SnoN (K-20), and c-Myc (9E-10) antibodies diluted 1:100 (Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. Vectastain ABC Elite kit was used for visualization of protein.

**Statistical Analysis.** A Student’s t test was used to compare mean cell proliferation of treated and control cells, and for the quantitative analysis of mRNA and protein expressions. Significance was defined as P < 0.05.
RESULTS

Effect of ECM on Primary Ovarian Culture Response to TGF-β. HOSEs and CSOCs cultured on collagen type I gel layers formed dense clumps of small rounded cells interconnected by long projections (Fig. 1). When plated at a higher density, cells formed multilayered sheaths. In contrast, HOSEs and CSOCs grown on plastic appeared flat with spindle-like projections (Fig. 1). Using CSOC monolayer cultures grown on plastic, we showed previously that CSOCs are resistant to TGF-β-mediated growth inhibition. To determine whether the presence of an ECM affects the response to TGF-β, we examined the sensitivity of HOSE and CSOC cultures plated on collagen I gels to TGF-β1 treatment. Consistent with our previous findings, 8 of 10 (80%) CSOC cultures did not demonstrate growth inhibition in response to TGF-β1, whereas the growth of 8 of 11 (73%) HOSE cultures was inhibited >15%. HOSE growth inhibition ranged between 10 and 50% (average 20 ± 21% inhibition; Fig. 2). In contrast, only 2 of 10 CSOCs were inhibited by <10%, and the majority were slightly stimulated (average 5 ± 10% stimulation) by TGF-β1 treatment (Fig. 2). The difference between the TGF-β growth response of HOSEs and CSOCs was statistically significant (P = 0.001).

Quantitative Analysis of TGF-β/Smad Signaling Components mRNA Levels in HOSEs and CSOCs. We performed a quantitative analysis of TGF-β receptors I and II, TGF-β signal transducers Smad2, 3, 4, 6, and 7, and transcriptional repressors SnoN and Ski mRNA expression levels using a RPA. Twenty HOSE and 20 CSOC cultures grown on collagen I matrices were used to compare basal mRNA expression levels between normal and malignant ovarian epithelial cell cultures. Statistically significant differences in basal expression between HOSE and CSOC cultures were not observed for any of the TGF-β receptors, Smads, or transcriptional repressors analyzed (Fig. 3).

Functional Analysis of TGF-β/Smad Signal Transduction. After Smad2 and 3 are phosphorylated directly by the ligand-activated TGF-β receptor tetramer, they oligomerize with Smad4 and are translocated into the nucleus. To determine whether Smad complex formation was altered in TGF-β-resistant CSOCs compared with sensitive HOSEs, we immunoprecipitated Smad complexes from 5 HOSE cultures and 5 CSOC cultures using an anti-Smad4 antibody, followed by immunoblotting with anti-Smad2 and 3 antibodies. Thus, only Smad2 and 3 associated with Smad4 were detected. The HOSE and CSOC cultures used for functional analyses were the 5 HOSEs that showed the greatest inhibition by TGF-β (20–50%) and the 5 CSOCs with the greatest resistance to TGF-β (stimulated by 5–25%). Treatment of these HOSEs and CSOCs with TGF-β for 0.5 h resulted in a statistically significant increase in Smad complex formation in both HOSE and CSOC cultures compared with unstimulated basal levels (Fig. 4). When compared with each other, TGF-β-induced complex formation was equivalent in HOSE and CSOC cultures, suggesting that TGF-β/Smad signal transduction was functional in CSOCs as well as HOSEs, despite the insensitivity of CSOCs to TGF-β-mediated growth inhibition.

Functional Analysis of the TGF-β/Smad Corepressors, Proto-oncoproteins SnoN and Ski, in HOSE and CSOC Cultures. Using mink lung epithelial cells transfected with SnoN and Ski, Sun et al. 
expression, resulting in a negative feedback to inactivate Smad complexes (16). We evaluated this response in TGF-β-treated HOSEs and CSOCs, and found that by 2 h of TGF-β stimulation, there was a significant and equivalent up-regulation of SnoN mRNA expression in both HOSEs and CSOCs (Fig. 6).

**Loss of c-myc Down-Regulation in Response to TGF-β.** Down-regulation of c-myc expression is one of the central antimitogenic responses to TGF-β (25). c-Myc is short-lived, and down-regulation of its mRNA by TGF-β results in a rapid loss of the protein (18). We assessed the effect of TGF-β treatment on c-myc expression in HOSEs and CSOCs by reverse transcription-PCR and Western blot analysis. TGF-β treatment rapidly decreased c-Myc protein and mRNA levels in HOSEs (Fig. 7). c-myc repression was sustained at least 8 h after TGF-β treatment in HOSEs. In contrast, c-myc mRNA levels were not significantly altered by TGF-β treatment, and TGF-β had no effect on c-Myc protein levels in CSOC cultures (Fig. 7). These results imply that the TGF-β/Smad signaling pathways are differentially regulated in ovarian cancer. TGF-β/Smad signaling remains functional in CSOC cultures and competent to induce some gene responses (SnoN re-expression); however, repression of c-myc expression and resulting cell cycle arrest is lost.

Fig. 4. TGF-β induces equivalent Smad complex formation in HOSEs and CSOCs. HOSEs (n = 5) and CSOCs (n = 5) were treated with TGF-β for 0, 0.5, and 2 h. Total cellular protein was immunoprecipitated with anti-Smad4 and immunoblotted with anti-Smad2 or 3. A, amount of Smad2/4 complex formation after TGF-β treatment. B, amount of Smad3/4 complex formation after TGF-β treatment. Each value represents an average of three independent experiments. Results are expressed as a ratio to untreated sample values. C, representative Western blot of Smad3/4 complex immunoprecipitated with anti-Smad4 and immunoblotted with anti-Smad3. * P < 0.05, ** P = 0.07, bars, ±SD.

Fig. 5. Western blot analysis of SnoN and Ski protein degradation after TGF-β treatment. A, TGF-β induced equivalent degradation of SnoN proto-oncoprotein in HOSEs and CSOCs. B, Ski protein levels are not regulated by TGF-β in HOSEs and CSOCs. HOSEs (n = 5) and CSOCs (n = 5) were treated with TGF-β for 0, 0.5, and 2 h. Each value represents an average of three independent experiments. Results are expressed as a ratio to untreated sample values. C and D, representative Western Blots of SnoN and Ski, respectively, are shown below each histogram. An equal amount of protein from each sample was analyzed. * P < 0.05; bars, ±SD.

Fig. 6. TGF-β induces de novo SnoN mRNA expression in both HOSE and CSOC. A, quantitative analysis of SnoN mRNA induction. There was no significant difference in the amount of SnoN up-regulation between HOSEs and CSOCs. HOSEs (n = 5) and CSOCs (n = 5) were treated with TGF-β for 0, 0.5, 2, and 4 h. RPA was used for detecting SnoN mRNA levels (B). G3PDH expression was used for normalization of sampling. Each value represents an average of three independent experiments. Results are expressed as a ratio to untreated sample values, *, P < 0.05; bars, ±SD.
c-myc mRNA levels decline to an imperceptible level in HOSEs after incubation with TGF-β (2). A significant difference in HOSE and CSOC sensitivity to TGF-β (P = 0.001) was observed using the collagen culture system. HOSEs were growth-inhibited by TGF-β, whereas CSOC cultures displayed resistance to TGF-β-mediated growth inhibition. Several immortalized ovarian cell lines have also been reported to be TGF-β resistant (36), whereas primary cultures established from ascites fluid appear to maintain sensitivity to TGF-β (37). Cells derived from ascites fluid may have distinctly different molecular properties than cells derived from solid tumors. One of the benefits of using primary cultures as investigative tools is that individual cultures reflect in vivo biological diversity.

Resistance to TGF-β growth arrest cannot be explained by functional mutations in TGF-β receptors or Smads, as these are infrequent in ovarian cancer (9–12). Therefore, we looked for alterations in the expression and/or function of the TGF-β/Smad pathway components. Comparing primary cultures of normal and malignant ovarian epithelia (HOSEs and CSOCs), we found no change in the expression levels of the TGF-β receptors I and II, consistent with our previous findings examining tumor tissue from primary ovarian cancers (25). In addition, we found the expression of the common-partner Smad4, the receptor-regulated Smads 2 and 3, and the antagonistic Smads 6 and 7 to be unaltered.

Complex formation between phosphorylated Smad 2 or 3 with Smad 4 is a key feature in TGF-β/Smad signal transduction. Missense mutations of interface amino acids that mediate complex formation have been identified in different tumors and have been shown to disrupt Smad complex formation in vitro (38). We analyzed Smad oligomerization by assessing the amount of complex formed after TGF-β treatment. TGF-β induced Smad2/4 and Smad3/4 complex formation in both normal and malignant ovarian cultures, without difference in the levels of complex formed.

The proto-oncoproteins Ski and SnoN act as Smad2 and 3 corepressors (13). The interaction of Smads with Ski and SnoN occurs under basal conditions, but the molecules dissociate after TGF-β stimulation because of TGF-β-induced degradation of Ski and SnoN proteins (17). Several hours after TGF-β treatment, a wave of SnoN expression is induced contributing to the termination of TGF-β response (17). A shift in the balance of Smads and corepressors within a cell, in principle, could change the outcome of TGF-β response in that cell. Ski and SnoN, found previously to be highly expressed in several human tumor cell lines including those derived from neuroblastomas and carcinomas of the stomach, vulva, and prostate (39), were shown to confer resistance to TGF-β or to cause oncogenic transformation when highly overexpressed in mink lung epithelial cells or Ba/F3 cells, respectively (40, 41). Therefore, we analyzed basal expression levels of Ski and SnoN, TGF-β-induced Ski and SnoN protein degradation, and the reinduction of SnoN mRNA in HOSEs and CSOCs. Our data show that overexpression of Ski and SnoN is not the cause of TGF-β resistance in ovarian cancer. Basal expression levels of Ski and SnoN mRNA in normal and malignant ovarian primary cell cultures were equivalent, and the same rate and amount of SnoN degradation was observed after TGF-β treatment. However, there was no significant degradation of Ski protein in either normal or malignant cultures induced by TGF-β. Considering that in a Ski-transformed mink lung cell line TGF-β was shown to induce Ski degradation (28), our data suggest that the effect of TGF-β on Ski degradation is cell-type dependent. Quantitative analysis of SnoN re-expression after TGF-β treatment showed equivalent induction of...
SnoN mRNA in both normal and malignant cultures. This result emphasizes that in malignant ovarian primary cultures that display an acquired resistance to TGF-β antiangiogenic effects, the major events in the TGF-β/Smad signaling pathway remain functional and capable of inducing responses in gene expression.

c-Myc down-regulation is a general feature of the TGF-β antiproliferative response (24). c-Myc is a ubiquitous promoter of cell growth and proliferation, and can either act as a transcriptional activator or repressor depending on the target gene (22). In this study, analysis of and proliferation, and can either act as a transcriptional activator or Smad complexes have also been approximately resulting in activating AP-1 or cAMP response element-regulatory complexes (45, 46). Smad complexes have also been demonstrated by unaltered Smad2/4 and 3/4 complex formation, SnoN degradation, and SnoN mRNA reinduction, the TGF-β signaling pathway in CSOC cultures remained competent to induce some gene responses but is unable to down-regulate c-myc. These data suggest that ovarian cancer cell resistance to TGF-β-mediated inhibition of cell proliferation.

Our results show that there is a selective loss of the TGF-β growth inhibition in primary cultures of malignant ovarian epithelium, which cannot be altered by alterations in the expression of the TGF-β/Smad pathway components or by the general failure of the TGF-β pathway. As demonstrated by unaltered Smad2/4 and 3/4 complex formation, SnoN degradation, and SnoN mRNA reinduction, the TGF-β signaling pathway in CSOC cultures remained competent to induce some gene responses but is unable to down-regulate c-myc. These data suggest that ovarian cancer cell resistance to TGF-β-mediated growth inhibition results from the loss of c-myc regulation. TGF-β resistance could also be caused by as yet unidentified component(s) of the TGF-β pathway, deficient formation of a Smad/c-Myc-TIE complex, or by involvement of other modulating pathways. Extensive evidence indicates that there is a multifaceted integration of signaling between Smad and mitogen-activated protein kinase pathways that is cell-type dependent. Among other effects, TGF-β has been shown to activate c-Jun NH2-terminal kinase or p38 kinases, possibly through Rho-like GTPases and TGF-β-activated kinase 1-binding protein 1/TGF-β-activated kinase 1 cascade, ultimately resulting in activating AP-1 or cAMP response element-regulatory complexes (45, 46). Smad complexes have also been shown to physically interact with Jun or activating transcription factor 2 complexes, their nuclear accumulation can be inhibited by Ras-activated extracellular signal-regulated kinases, and they can undergo activating phosphorylation by c-Jun NH2-terminal kinase (45, 46). Therefore, the activity of these other pathways will influence the outcome of a TGF-β response in a given cell at a given time. An important notion remains that, in addition to autocrine tumor suppressing activities, TGF-β exhibits autocrine and paracrine tumor-promoting activities such as increased tumor cell invasion (47), stimulation of stromal proliferation, angiogenesis, fibrosis, and immunosuppression (48). Therefore, ovarian cancer cells likely derive a selective advantage by keeping a functional tumor-promoting TGF-β signaling pathway while repressing TGF-β antiproliferative responses.

REFERENCES


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