Inability of Transforming Growth Factor-β to Cause SnoN Degradation Leads to Resistance to Transforming Growth Factor-β–Induced Growth Arrest in Esophageal Cancer Cells

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Abstract
It is well established that loss of a growth inhibitory response to transforming growth factor-β (TGF-β) is a common feature of epithelial cancers including esophageal cancer. However, the molecular basis for the abrogation of this key homeostatic mechanism is poorly understood. In esophageal cancer cell lines that are resistant to TGF-β–induced growth inhibition, TGF-β also fails to decrease transcription of c-myc despite the presence of functional signaling components. Consequently, to gain a better understanding of the mechanisms leading to resistance to TGF-β–induced growth arrest, the basis for the inability to decrease c-myc transcription was investigated. Regardless of sensitivity to TGF-β–induced growth arrest, TGF-β enhanced the ability of Smad3-protein complexes to bind c-myc regulatory elements. However, in a growth inhibition–resistant esophageal cancer cell line, the Smad3-protein complexes contained the SnoN oncoprotein. Furthermore, in esophageal cancer cell lines that are resistant to TGF-β–induced growth arrest, TGF-β does not cause degradation of SnoN. Analyses of the effect of modulating SnoN expression in both growth inhibition–sensitive and growth inhibition–resistant cell lines showed that degradation of SnoN is a prerequisite for both TGF-β–induced repression of c-myc transcription and growth arrest. The data indicate that SnoN-Smad3 complexes do not cause repression of c-myc transcription but rather prevent functionality of active repressor complexes. Thus, these studies reveal a novel mechanism for resistance to TGF-β–induced growth inhibition in esophageal cancer, namely the failure to degrade SnoN. In addition, they show that SnoN can block TGF-β repression of gene transcription.

Introduction
Esophageal cancer has one of the lowest survival rates of any cancer. In fact, only primary cancers of the liver, biliary tract, and pancreas have higher mortality rates. The bleakness of the scenario is compounded by the fact that the occurrence of esophageal cancer, particularly adenocarcinoma of the esophagus, is increasing (1). Although improvements in staging combined with multimodality therapy and improvements in surgical approaches have provided increased survival in a subset of esophageal cancer patients (2), it seems unlikely that additional refinements in current treatment modalities will provide a significant increase in long-term survival. Consequently, increased understanding of the molecular basis for the development of esophageal cancer and identification of new therapeutic targets is paramount for improving the prognosis of patients with these deadly cancers.

Transforming growth factor-β (TGF-β) is a pleiotropic cytokine that plays a central role in maintaining epithelial homeostasis, and altered responses to TGF-β are widely associated with epithelial cancers (3). In normal epithelial cells, TGF-β induces a reversible arrest in G1 (4). In contrast, cancers of epithelial cell origin tend to be resistant to the growth inhibitory effect of TGF-β (4). Although many tumors have lost components of the TGF-β pathway, resistance to the growth inhibitory effect frequently occurs in the presence of a functional signaling pathway (5). Because cancers with functional pathways and resistance to growth inhibition seem to be more aggressive (6), it is important to ascertain the molecular basis for resistance to TGF-β–induced growth inhibition in epithelial cancers.

TGF-β causes epithelial cells to arrest in G1 by eliciting a rapid decrease in expression of c-myc, which in turn leads to increased expression of the cdk inhibitors p21 or p15 (7–9). Studies with breast cancer cells showed that abrogation of the growth inhibitory response was the result of a selective loss in the ability of TGF-β to down-regulate expression of c-myc (10). The central role of failure to decrease c-myc in the loss of the growth inhibitory response during tumorigenesis in epithelium is supported by studies in oral pharyngeal, ovarian, and esophageal cancers (11–13). However, despite functional TGF-β pathways, primary ovarian cancer cells and several esophageal cancer cell lines both failed to be growth inhibited and decrease c-myc expression in response to TGF-β. Mechanistically, TGF-β regulation of c-myc expression occurs at the transcriptional level. In breast cancer–derived cells that are sensitive to the growth inhibitory effect of TGF-β, TGF-β–induced phosphorylation of Smad3 results in translocation of a Smad3-p107 complex to the nucleus (14). This complex binds to a region in the promoter, the TGF-β inhibitory element (TIE), which contains both Smad and E2F4 binding elements and represses transcription (14–16).

SnoN (Ski-like novel gene) is a member of the Ski family of proto-oncogenes. It plays a role in both development and tumorigenesis (17, 18). The absence of SnoN is lethal for embryonic mice. However, modulation of SnoN expression seems to have cell type–specific effects. For example, increased SnoN expression induces fibroblast transformation (19). In contrast, mice that are SnoN heterozygotes have decreased expression of SnoN and develop lymphomas spontaneously (17). These findings suggest that SnoN may function as either a tumor promoter or tumor suppressor.
depending on the cell type. Regardless, the effect of SnoN depends on interaction with Smad proteins (20, 21). Following TGF-β treatment, activated Smads target SnoN to the proteasome for degradation (22, 23). The resulting loss of SnoN from Smad3-protein complexes permits activation of transcription (24, 25). Although SnoN and Ski play different roles in development and tumorigenesis, overexpression of either one leads to resistance to TGF-β–induced growth arrest (22, 26). In the present study, the role of SnoN in abrogation of TGF-β–induced growth arrest and tumor suppression was investigated using a model system of esophageal cancer. The data reveal both a novel mechanism for resistance to TGF-β–induced growth arrest and loss of repression of c-myc transcription as well as providing new insight into the mechanism of SnoN regulation of the effects of TGF-β on gene transcription.

Materials and Methods

Cell lines. OE33 and OE21, which were derived from an adenocarcinoma and a squamous cell carcinoma of the esophagus, respectively, were purchased from the European Collection of Cell Cultures. SEG-1, which was derived from an adenocarcinoma in the context of Barrett’s esophagus, was kindly provided by Dr. Andrew Joe (Columbia University, New York, NY). All cell lines were maintained in RPMI 1640 supplemented with 10% fetal bovine serum.

Cell growth. Briefly, 5 × 10^4 to 10 × 10^4 cells were plated in 96-well plates and treated with or without TGF-β (100 nmol/L). On day 7, cell growth was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (27).

Immunoblotting and immunoprecipitation. Total cellular extracts were obtained by washing the cells twice with ice-cold PBS (pH 7.4) and scraping the cells in ice-cold lysis buffer (1% PBS, 1% Igepal CA-630, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with 1 mmol/L phenylmethylsulfonyl fluoride, 1× protease inhibitor cocktail, 1× phosphatase inhibitor cocktail I, and 1× phosphatase inhibitor cocktail II (all from Sigma-Aldrich, St. Louis, MO). Cytoplasmic and nuclear extracts were prepared using NE-PER (Pierce, Rockford, IL). Proteins were quantified using the Bio-Rad protein assay (Bio-Rad, Hercules, CA), and 30 to 50 μg were used per sample for Western blotting. Samples were resolved by SDS-PAGE, transferred to polyvinylidene difluoride (Bio-Rad), and blocked for 1 hour with 5% nonfat dry milk (Bio-Rad) in Tris-buffered saline containing 0.05% Tween 20 (TBST; Sigma-Aldrich). The blots were incubated with the primary antibodies overnight at 4°C, washed thrice with TBST for 10 minutes, and incubated with appropriate secondary horseradish peroxidase–conjugated antibody (Zymed, South San Francisco, CA) for 1 hour at room temperature. Blots were then washed four times with TBST for 10 minutes each, developed with ECL plus (Amersham Biosciences, Inc., Piscataway, NJ), and exposed to X-Omat blue film (Kodak, Rochester, NY).

For immunoprecipitation, total cellular extracts were prepared in HKMG buffer [10% glycerol, 12.5 mmol/L HEPES (pH 7.6), 50 mmol/L KCl, 0.1 mmol/L EDTA, 1 mmol/L DTT] as described (10). Cellular extract (1 mg/mL) and either anti-Smad3 (Zymed) or anti-HA (12CA5, Roche, Indianapolis, IN) at 2 μg/mL were incubated overnight at 4°C. The PCRs were cactcagactgttggaagg (forward) and cacagaagctgacctcc (reverse). PCR products were sequenced directly in an ABI Prism 377 DNA sequencer in the Nucleic Acids Core Facility, Massey Cancer Center.

Results

SnoN-Smad3 complexes associate with transforming growth factor-β inhibitory element in growth inhibition–resistant esophageal cancer cells. TGF-β causes epithelial cells to arrest in G1 by eliciting a rapid decrease in expression of c-myc, which in turn leads to increased expression of the cdk inhibitors p21 or p15 (4). However, other reports have suggested that down-regulation of c-myc may not be a prerequisite for TGF-β–mediated growth inhibition in all cell types (28). To address the role of c-myc in the growth inhibitory response of esophageal epithelial cells, the effect of TGF-β on c-myc expression was assessed. OE33, which is growth-inhibited by TGF-β, rapidly decreases both c-myc mRNA (13) and protein levels (Fig. 1) following treatment with TGF-β. In contrast, TGF-β failed to decrease either c-myc mRNA (13) or protein levels (Fig. 1) in the cell lines that were not growth inhibited. However, the components of the TGF-β pathway are present and functional in each of the cell lines as indicated by phosphorylation of Smad2 (Fig. 1) and the ability to activate TGF-β–responsive promoters (13). These findings suggest that failure to regulate c-myc expression plays a key role in resistance to TGF-β–induced growth inhibition in esophageal cancer cells.

Because the failure to decrease expression of c-myc seems to be central to resistance to TGF-β–induced growth inhibition in several epithelial cancers, regulation of c-myc expression was evaluated in both growth inhibition–sensitive OE33 and growth inhibition–resistant SEG-1 and OE21. Other investigators identified an element, the TIE, 5′ to the c-myc gene that is required for TGF-β–induced repression of c-myc transcription (14–16). In cells

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whereas it has no effect on SnoN-Smad3 complexes in either SEG-1 or OE21 (Fig. 3A). Furthermore, TGF-β treatment induces a decrease in SnoN expression in OE33, but not SEG-1 or OE21 (Fig. 3B). Thus, the composition of the complexes capable of binding the TIE mirrors the changes in both Smad3-SnoN complexes and SnoN expression.

One possibility suggested by studies in melanoma (29) is that development of esophageal cancer is associated with alterations in subcellular localization of SnoN. However, as shown in Fig. 3C, SnoN is localized to the nucleus regardless of sensitivity to TGF-β-induced growth arrest. Consequently, neither the development of esophageal cancer nor the failure to degrade SnoN results from alterations in subcellular localization. The presence of Smad3-SnoN complexes in the absence of TGF-β treatment is due to the interaction between nuclear Smad3 and SnoN. This finding is consistent with the results of other studies demonstrating both the presence of Smad3 in both the nucleus and cytoplasm in the absence of TGF-β stimulation (14) and the ability of transacted Smad3 to form complexes with SnoN in the absence of TGF-β treatment (22). A second possible explanation is that the failure to degrade SnoN is due to mutations in the gene. Smad3-APC–dependent degradation of SnoN depends on the presence of a destruction or D-box and lysines for ubiquitin attachment (22, 23). The essential lysines are 440, 446, and 449 (22). To ascertain if mutation in these elements could be responsible for failure to degrade SnoN, a 1.1 kb region containing these elements was amplified by PCR using cDNAs derived from OE33, SEG-1, and OE21 (cf. Materials and Methods). Nucleotide sequence analysis showed that both the D-box and lysines were present and no mutations were observed in the entire region (data not shown). Taken together, these findings indicate that the inability of TGF-β to cause a decrease in SnoN expression is a consequence of a specific defect in protein turnover or degradation.

**Decreased SnoN expression is a prerequisite for transforming growth factor-β inhibition of c-myc transcription.** The correlation between the failure of TGF-β to decrease SnoN expression and resistance to growth inhibition suggests a causal link between regulation of SnoN and c-myc. Several studies showed that the rapid decrease in SnoN expression following TGF-β treatment is the result of Smad3 targeting of SnoN to the cytoplasm in the absence of TGF-β stimulation (14) and the finding is consistent with the results of other studies demonstrating both the presence of Smad3 in both the nucleus and cytoplasm in the absence of TGF-β stimulation (14) and the ability of transacted Smad3 to form complexes with SnoN in the absence of TGF-β treatment (22). A second possible explanation is that the failure to degrade SnoN is due to mutations in the gene. Smad3-APC–dependent degradation of SnoN depends on the presence of a destruction or D-box and lysines for ubiquitin attachment (22, 23). The essential lysines are 440, 446, and 449 (22). To ascertain if mutation in these elements could be responsible for failure to degrade SnoN, a 1.1 kb region containing these elements was amplified by PCR using cDNAs derived from OE33, SEG-1, and OE21 (cf. Materials and Methods). Nucleotide sequence analysis showed that both the D-box and lysines were present and no mutations were observed in the entire region (data not shown). Taken together, these findings indicate that the inability of TGF-β to cause a decrease in SnoN expression is a consequence of a specific defect in protein turnover or degradation.

**Growth inhibition–resistant esophageal cancer cell lines fail to degrade SnoN.** To determine if the level of SnoN in TIE-associated complexes reflects changes in SnoN-Smad3 complexes in the cell lines, cell extracts from untreated and TGF-β–treated cells were immunoprecipitated with anti-Smad3. In OE33, TGF-β treatment decreases the level of SnoN associated with Smad3, whereas it has no effect on SnoN-Smad3 complexes in either SEG-1 or OE21 (Fig. 3A). Furthermore, TGF-β treatment induces a decrease in SnoN expression in OE33, but not SEG-1 or OE21 (Fig. 3B). Thus, the composition of the complexes capable of binding the TIE mirrors the changes in both Smad3-SnoN complexes and SnoN expression.

Figure 1. Resistance to TGF-β–induced growth inhibition correlates with failure to down-regulate c-myc expression. A, all cell lines were incubated with 100 pmol/L TGF-β and an MTT assay was done on day 7 of culture. Results are expressed as the ratio of absorbance of TGF-β–treated to untreated (control) cultures. Columns, mean from three experiments; bars, SE. B, cells were cultured for 4 hours in the presence (+) or absence (−) of 100 pmol/L TGF-β and expression of indicated proteins was determined by Western analysis. C, cells were cultured for 15 minutes in the presence (+) or absence (−) of 100 pmol/L TGF-β and expression of indicated proteins was determined by Western analysis.

![Figure 1](cancerres.aacrjournals.org)
proteasome for degradation (22, 23). Consequently, if SnoN degradation is critical for decreased c-myc transcription, it would be predicted that TGF-β regulation of c-myc expression would also be proteasome dependent. To address this, OE33 cells were treated with TGF-β in the presence or absence of MG132, an inhibitor of proteasome activity. As shown in Fig. 4A, TGF-β induced decrease in expression of both c-myc and SnoN required proteasome activity. Interestingly, SnoN degradation occurs before the decrease in c-myc expression, further suggesting a causal link.

To ascertain directly if altered SnoN expression is required for TGF-β regulation of c-myc, SnoN expression was modulated in growth inhibition–sensitive OE33 as well as the growth inhibition–resistant cell lines OE21 and SEG-1. TGF-β–mediated degradation of SnoN has been shown to depend on a Smad3 binding site, a D-box, and lysines at positions 440, 446, and 449 (22). Therefore, we created expression plasmids containing either a truncated form of SnoN that has both a Smad3 binding site and a D-box but does not have the lysines (T-SnoN) or a SnoN gene in which all three lysines were mutated to arginine. OE33 cells were transiently transfected with one of the SnoN expression plasmids, a reporter plasmid containing the firefly luciferase gene under control of a region of the c-myc promoter and a reference plasmid containing the Renilla luciferase gene under control of the thymidine kinase promoter (Fig. 4B). In OE33 that were transfected with an empty expression vector in lieu of a SnoN expression vector, TGF-β treatment decreased expression of firefly luciferase, whereas transfection with either SnoN expression vector inhibited TGF-β down-regulation of firefly luciferase, indicating that SnoN can
block the ability of TGF-β to decrease c-myc transcription (Fig. 4B). The effect of SnoN expression on TGF-β regulation of c-myc was confirmed by decreasing SnoN expression in OE21 and SEG-1. Transient transfection with SnoN siRNAs decreased both SnoN expression and restored TGF-β down-regulation of c-myc expression in OE21 (Fig. 4C). In SEG-1, transient transfection with SnoN siRNAs also decreased SnoN expression albeit somewhat less efficiently than in OE21 (Fig. 4C). Taken together, these studies indicate that SnoN degradation is a prerequisite for a central response in TGF-β-induced growth arrest, namely decreased c-myc transcription.

**Failure to degrade SnoN disrupts the transforming growth factor-β growth arrest program.** To address more fully the role of modulation of SnoN expression in the TGF-β growth arrest program, OE33 was stably transfected with an expression vector encoding a truncated form of SnoN, which is not degraded in response to TGF-β (Fig. 5A). Clones of OE33 expressing varying levels of the truncated SnoN were isolated and assayed for growth in the presence and absence of TGF-β (Fig. 5B). Compared to parental OE33, the SnoN transfectants were significantly more resistant to TGF-β–induced growth arrest (Fig. 5B). The sensitivity to growth inhibition was paralleled by the ability to decrease c-myc expression (Fig. 6A). Four hours after treatment with TGF-β, the level of c-myc was significantly decreased in cultures of parental OE33, but not in cultures of pooled SnoN transfectants. Furthermore, in parental OE33, 10 pmol/L TGF-β was sufficient for maximal decrease in c-myc expression, whereas a concentration as high as 100 pmol/L TGF-β did not cause a comparable decrease in c-myc expression in SnoN transfectants.

Because the primary model for Ski family modulation of Smad-mediated transcription is that they repress transcription by recruiting histone deacetylase to the promoter (18), it was not clear how SnoN could inhibit TGF-β–induced repression of transcription. One possibility is that the failure to degrade SnoN inhibits the ability of Smad3 complexes to bind the TIE. To address this issue, DNA precipitations using the c-myc TIE were done with both parental OE33 and truncated SnoN transfectants. In both cell lines, TGF-β induced a similar increase in binding of Smad3 complexes to the TIE (Fig. 6B). However, the level of endogenous SnoN in the complexes decreases relative to the level of Smad3, whereas the level of transfected SnoN in the complexes mirrors the increase in Smad3. To confirm the observation that SnoN does not inhibit Smad3 association with the TIE, DNA precipitations were also done using OE33 cells treated with the proteasome inhibitor MG132 to block degradation of SnoN. The TGF-β–induced increase in Smad3 association with the TIE is similar in both untreated and MG132-treated OE33 (Fig. 6C). In the MG132-treated but not in untreated OE33,
the level of SnoN in TIE-associated complexes increases parallel to the increase in Smad3. These data indicate that SnoN does not prevent Smad3 complexes from binding to the TIE, suggesting that Smad3-SnoN association prevents functionality of a repressor complex.

Discussion

The TGF-β pathway plays a dual role in the development and progression of epithelial cancers. In normal epithelial cells, treatment with TGF-β leads to a reversible arrest in G1 (4). The observation that a key component of TGF-β signaling, Smad4 or DPC4, was absent in pancreatic cancers lead to the idea that in normal epithelium, TGF-β-suppressed tumor development and absence of components of the pathway was involved in development of cancer (30). This idea was reinforced by the observation that the subset of colon cancers with microsatellite instability also lacked components of the pathway (31, 32). Additional observations put a twist into the TGF-β story. The prognosis for individuals with colon cancer lacking TGF-βRII turned out to be better than that for individuals with colon cancers that had intact TGF-β pathways (33, 34). Similarly, studies with breast cancer cells revealed that cancers with an intact TGF-β pathway were more aggressive than those with a defective pathway (6). Ultimately, these observations revealed the paradox of the TGF-β pathway. Once an epithelial cell becomes resistant to the growth inhibitory effect and embarks on the pathway to cancer, it becomes increasingly susceptible to TGF-β responses that promote metastasis and invasion (3, 5). Thus, a central issue in understanding the role of TGF-β in development and progression of epithelial cancers becomes understanding the genetic and epigenetic events responsible for resistance to growth inhibition.

Analysis of both primary tumors and cell lines showed components of the TGF-β pathway are generally present and functional in esophageal cancers (13, 35). However, as is the case with many epithelial cancers, esophageal cancer cell lines are for the most part resistant to TGF-β-induced growth inhibition (13). The cell lines that failed to be growth inhibited by TGF-β also failed to decrease c-myc transcription (13). In this regard, abrogation of TGF-β repression of c-myc transcription seems to be the linchpin in the growth arrest program. For example, TGF-β growth inhibition–resistant breast cancer cells selectively lose the ability to repress c-myc transcription in response to TGF-β (10). Similarly, primary ovarian cancer cells are both resistant to TGF-β–induced growth arrest and unable to down-regulate c-myc expression (12). Thus, ascertaining the mechanism responsible for loss of this key gene response will provide insight into the cellular lesion responsible for resistance to growth inhibition.

Several groups have investigated the mechanism by which TGF-β represses c-myc transcription (14–16). There is general agreement that repression is mediated by an element, the TIE, which contains both a Smad and E2F binding site as well as the fact that treatment with TGF-β enhances the ability of Smad3 to interact with the TIE. There is some controversy regarding the effect of TGF-β on recruitment of p107 to the complex. In two studies, TGF-β treatment did not lead to an increase in the association of p107 with the TIE (15, 16), whereas in another study TGF-β treatment did lead to an increase in the association of p107 with the TIE (14). However, in the latter study, only the interaction between the TIE and Smad3-containing complexes was evaluated. Because the TIE contains an E2F binding element and p107 can bind to E2F4 (14), it is possible that p107 interaction with the TIE in total cell extracts can be Smad3 independent. In the growth inhibition–sensitive esophageal cancer cell line OE33, TGF-β increases the ability of Smad3 to bind the TIE, but it has no effect on p107 binding. The same is true of the growth inhibition–resistant esophageal cancer cell lines SEG-1 and OE21, raising the possibility that additional proteins are present in the Smad3 complex that prevent it from functioning properly. Significantly, following treatment with TGF-β, Smad3 remains associated with the SnoN oncprotein in SEG-1 as well as in OE21. The presence of SnoN in Smad3 complexes regardless of treatment with TGF-β reflects the fact that in growth inhibition–resistant cell lines, TGF-β does not induce a decrease in SnoN expression. SEG-1 both expresses a higher level of SnoN than OE33 and seems to phosphorylate Smad3 less efficiently. It is unlikely that these differences account for the failure to degrade SnoN because OE21 expresses less SnoN than OE33 and seems to phosphorylate Smad3 as efficiently as OE33.

Our observation that in TGF-β growth inhibition–resistant cell lines SnoN remained associated with Smad3 on the myc TIE suggested that the failure to degrade SnoN is responsible for the loss of a key event in the TGF-β growth arrest program, namely decreased transcription of c-myc. The validity of this hypothesis was confirmed by manipulation of SnoN expression in growth inhibition–sensitive and growth inhibition–resistant cell lines. Introduction of a nondegradable SnoN into growth inhibition–sensitive OE33 abrogates both TGF-β–induced growth arrest and down-regulation of c-myc. On the other hand, decreasing SnoN expression in both OE21 and SEG-1 restores TGF-β repression of c-myc transcription. Thus, these studies identify a novel mechanism for resistance to TGF-β–induced growth arrest in esophageal cancer.

In light of the proposed mechanism of SnoN regulation of TGF-β gene responses, the observation that SnoN expression affects c-myc expression was unexpected. The primary model for SnoN regulation of TGF-β regulation of gene expression, which is based on observations regarding Ski binding partners, is that Ski/SnoN binding to N-Cor recruits histone deacetylase to the promoter causing deacetylation and transcriptional repression (18). Although this mechanism is consistent with previous studies demonstrating that SnoN blocks TGF-β activation of gene transcription (24, 25), it is difficult to reconcile this model with the observation that SnoN blocks TGF-β repression of c-myc transcription. However, it has been shown that Ski can block TGF-β activation of transcription even when its N-Cor binding site is mutated (36). An alternative model suggests that Ski interferes with the formation of Smad3-Smad4 heteromers (37). In this model, the recruitment of N-Cor may not be necessary for interference with Smad-mediated regulation of transcription. However, the effect of Ski on Smad3-Smad4 heteromer formation is controversial because a subsequent study showed that Ski does not block Smad3-Smad4 heteromer formation and binding to either Smad is sufficient for Ski function (38). In addition, a recent study indicated that Ski can block TGF-β repression of transcription by forming Smad complexes that are more stable and have a higher affinity for DNA than functional complexes that do not contain Ski (39). Our observation that SnoN does not affect the ability of Smad3 complexes to bind the TIE supports the latter model. Nonetheless, it remains possible that SnoN blocks TGF-β repression of c-myc transcription either by recruiting proteins that interfere with the functionality of an intact repressor complex or by preventing recruitment of a yet to be
identified protein that is essential for TGF-β repression of c-myc transcription. The present study shows that abrogation of TGF-β-induced SnoN degradation blocks repression of c-myc transcription and increases resistance to growth inhibition. The findings raise the possibility that development of esophageal cancer can be associated with a specific defect in proteasome-mediated protein degradation. They also suggest that appropriate, targeted modulation of proteasome activity rather than total inhibition of proteasome function may prove effective in treatment of a subset of esophageal cancer. Our observations also support the hypothesis that increased expression of SnoN promotes tumorigenesis by inhibiting the growth-suppressive effect of TGF-β, suggesting that in the subset of squamous cell esophageal cancers with 3q26 amplification and high levels of SnoN expression, modulation of SnoN expression could be of therapeutic benefit.

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