The Human Papilloma Virus E7 Oncoprotein Inhibits Transforming Growth Factor-β Signaling by Blocking Binding of the Smad Complex to Its Target Sequence*

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The human papillomavirus (HPV) oncprotein E7 is implicated in the etiology of cervical cancer associated with infection by HPV. HPV-positive cells develop resistance to TGF-β growth inhibitory activity through the inhibition of hypophosphorylation of pRb by papillomavirus type 16 E7 oncoprotein. In this study, we examined whether E7, in addition to its well known effects on pRb, might directly target the Smad proteins that mediate TGF-β signaling. Here, we show that E7 significantly blocks both Smad transcriptional activity and the ability of TGF-β to inhibit DNA synthesis. We found that E7 interacts constitutively with Smad2, Smad3, and Smad4. Confocal microscopic studies confirm that E7 and Smads co-localize in vivo. Using a canonical Smad DNA binding sequence, we found that E7 blocks Smad3 binding to its target sequence on DNA. These results suggest that suppression of Smad-mediated signaling by E7 may contribute to HPV-associated carcinogenesis.

Cervical cancer is the second most common cause of cancer death in women worldwide. During the year 2000, over 4800 women died as a result of the disease (1). Development of cervical cancer is highly associated with infection by high risk human papillomavirus (HPV) types such as HPV-16 or HPV-18, whereas low risk HPV types like HPV-6 are associated with benign genital warts. The high risk HPVs encode two transforming genes, E6 and E7. Both E6 and E7 interfere with key elements in the cell cycle control machinery and as a result, induce cell cycle progression in cells that normally would be quiescent. Whereas E6 mediates the accelerated proteosomal degradation of the p53 tumor suppressor, E7 has been shown to bind to and destabilize the product of the retinoblastoma susceptibility gene pRb (pRb) and interferes with the cyclin-dependent kinase inhibitor p21Cip (2–3).

TGF-β inhibits the proliferation of most epithelial and lymphoid cells. This negative regulation of cellular proliferation by TGF-β has been shown to constitute a tumor suppressor pathway (4). The arrest of cell proliferation by TGF-β is associated with increased expression or activity of several cyclin-dependent kinase inhibitors, including p15Ink4b, p21Waf/Cip, and p27Kip1 (5–8). The increase in p15Ink4b, p21Waf/Cip, and p27Kip1 activity results in a decrease in G1 Cdk activity, which maintains pRb in its hypophosphorylated state (9). Hypophosphorylated pRb sequesters E2F factors needed for progression into S phase of the cell cycle, thereby leading to cell cycle arrest in G1 (5). Smad2 and Smad3 have been identified as direct downstream mediators of TGF-β signaling (10). Receptor-mediated phosphorylation of these Smads induces their association with the shared partner Smad4, followed by translocation into the nucleus where these complexes activate transcription of specific genes (11, 12).

In this study, we demonstrate that the HPV type 16 E7 protein blocks the ability of TGF-β to activate transcription and inhibit cell proliferation. We show that E7 binds to Smad2, Smad3, and Smad4. Furthermore, we demonstrate that E7 blocks the interaction of the Smad complex with the Smad DNA binding element, CAGA, causing inhibition of TGF-β signal transduction. In E7-expressing MvILu cells, we found that the effect of TGF-β on Cdk2 or Cdk4 synthesis is abrogated, and phosphorylation of pRb is increased. From these results, we suggest that the direct inhibition of Smad-mediated TGF-β signaling by E7 may contribute to HPV-associated carcinogenesis.

MATERIALS AND METHODS

Constructs—FLAG-tagged Smad3 deletion constructs were generated by polymerase chain reaction using a proofreading polymerase and subcloned into the PCMV-FLAG vector. All polymerase chain reaction-generated products were sequenced using the dyeodeoxynucleotide method. E7 expression lines were described previously (14). For cell proliferation assay, E7-expressing cells were plated in 24-well dishes at a density of 5 × 10⁴ cells per well in 0.5 ml of assay medium (Dulbecco’s modified Eagle’s medium/0.2% fetal bovine serum). After incubating for 22 h in the presence or absence of TGF-β, cells were pulse-labeled with 0.5 μCi of [3H]thymidine for 2 h at 37 °C. Cells were fixed, trypsinized, solubilized, and transferred to scintillation vials to measure radioactivity as described previously (15).

Cell Culture, Transfection, and Reporter Assays—Cell lines were maintained in minimal essential medium supplemented with 10% fetal bovine serum. HepG2 cells were transfected with 3TP-Lux (16), 4xSBE-luc (17), p-800 lux (18), in six-well plates using Lipofectin (Invitrogen, Rockville, MD) according to the manufacturer’s instructions. After transfection, cells were treated with 5 ng/ml TGF-β1 for 24 h in media. All assays were performed in triplicate and represented as mean ± S.E. of three independent transfections.

Western Blots and Immunoprecipitation—HepG2 cells were transiently transfected with the indicated plasmids. After 24 h, cells were
FIG. 1. E7 represses TGF-β1-induced transcriptional activation. E7 was co-transfected into HepG2 cells with either 3TP-Lux (A) or SBE4-luc (B). Luciferase activity was measured 24 h after TGF-β1 stimulation. All values represent the mean (±S.E.) of triplicate measurements from one representative transfection. *, p < 0.05 (t test).

switched to 0.2% serum overnight, and induced 5 ng/ml TGF-β1 for 2 h and then whole cell extracts were prepared as described previously (19). Extracts were separated by SDS-PAGE followed by electrotransfer to nitrocellulose membranes and probed with polyclonal or monoclonal antisera, followed by hors eradish peroxidase-conjugated anti-rabbit, anti-mouse, anti-goat IgG, respectively, and visualized by chemiluminescence according to the manufacturer’s instructions (Pierce Chemical Co., Rockford, IL). Immunoprecipitation were carried out by incubation with antibody for 1 h. After immunoprecipitates were washed with the buffer containing 100 mM NaCl and 75 mM KCl, Western blots were prepared.

For cell cycle signal protein detection, Mv1Lu cells were lysed in the same extraction buffer above after 24-h TGF-β1 treatment (5 ng/ml, twice at 0 and 12 h). Protein concentrations were determined using Bradford method (Bio-Rad Laboratories, Hercules, CA). Samples of total protein lysate were subjected to SDS-PAGE and then transferred to a nitrocellulose membrane. The following commercial antibodies were used: for p21, p27, and p15 (Santa Cruz Biotechnology Inc., Santa Cruz, CA) and for β-actin (Sigma-Aldrich, St. Louis, MO).

GST Pull-down Assay—The coding region for Smad2, Smad3, or Smad4 was PCR-amplified and subcloned into the TOPO vector (Invitrogen Corp., Carlsbad, CA). These plasmids were used as templates for RNA synthesis by T7 RNA polymerase followed by translation in rabbit reticulocyte extracts (Promega Corp., Madison, WI). GST-E7 fusion protein expressed in E. coli was grown and partially purified by adsorption to glutathione-Sepharose beads in the presence of the detergent N-laurylsarcosine (Sarkosyl) and Triton X-100. Samples of each protein (0.5–1.0 μg) bound to Sepharose were preincubated with ethidium bromide (40 μg/ml) for 30 min. Then the samples were shaken for 1 h at room temperature with 5–10 μl of [32P]methionine-labeled in vitro translated Smad proteins. The beads were washed four times in NETN buffer (20 mM Tris (pH 8.0), 100 mM NaCl, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 1.0 mM DTT, 1.0 mM EDTA, 0.5 mM PMSF, 1 μg of both leupeptin and aprotinin per milliliter). After digestion with streptavidin-coated magnetic beads, the beads were washed three times in NETN buffer before fractionation on 4% nondenaturing polyacrylamide gel and run in 0.5× Tris borate-EDTA buffer. After electrophoresis the gel was dried and autoradiographed.

RESULTS

Transcriptional Repression of a TGF-β-responsive Gene by HPV E7—To examine the effect of E7 on TGF-β1-induced transcriptional activation, we co-transfected HepG2 cells with an E7 expression construct along with the TGF-β1-responsive 3TP-lux reporter construct or the SBE4-luc reporter construct that contains four SBE (Smad binding element) sites in tandem (17). Introduction of E7, but not E6, repressed the TGF-β1-dependent activities of these reporter gene constructs (Fig. 1, A and B), suggesting that E7 represses TGF-β1-induced transcriptional activation. Specifically, the repression of the SBE4-luc reporter construct or the SBE4-luc reporter construct that contains four SBE (Smad binding element) sites in tandem (17). Introduction of E7, but not E6, repressed the TGF-β1-dependent activities of these reporter gene constructs (Fig. 1, A and B), suggesting that E7 represses TGF-β1-induced transcriptional activation.

To examine whether E7 blocks the ability of TGF-β to inhibit G1/S transition and DNA synthesis in stable, retrovirus-infected Mv1Lu cells, TGF-β1-induced DNA synthesis of control Mv1Lu cells (Mv1Lu-LXSN). However, expression of E7 (Mv1Lu-LXSN-E7)
E7 blocks the TGF-β growth inhibitory activity. A, thymidine incorporation as a function of varying concentrations of TGF-β1 (expressed as a percentage of the untreated control). E7-expressing Mv1Lu cells and control cells were treated with varying concentrations of TGF-β1 as indicated. After 20 h of TGF-β1 treatment, the Mv1Lu cells were pulsed with [3H]thymidine and harvested 3 h later. All values represent the mean (± S.D., n = 3). B, E7 protein expression in Mv1Lu cells. C, Western blots for cell cycle proteins in E7-expressing Mv1Lu cells and control cells treated with or without TGF-β1 treatment. The proteins were detected by using anti-pRb, anti-Cdk4, anti-Cdk2, anti-E7, and anti-β-actin antibodies. D, control and E7-expressing Mv1Lu cells were treated with TGF-β1 (5 ng/ml), and cell lysates were prepared, followed by immunoblotting analysis. The proteins were detected by using the anti-p15 and -p27 antibodies.

Although p21 has been suggested to mediate TGF-β growth inhibition, its level in Mv1Lu cells were found to be low and were not affected by TGF-β (24 and data not shown).

E7 Interacts with Smads—Because E7 interferes with Smad-mediated transactivation, we examined whether Smad2, Smad3, or Smad4 physically interacts with E7 in vivo. Interaction of Smads with E7 was tested by co-immunoprecipitation of Smad2, Smad3, or Smad4 and E7 from HepG2 cells transfected with Smad expression constructs. Smad2, Smad3, and Smad4 each were detected in anti-E7 immunoprecipitates by protein immunoblotting with antibody to FLAG-tagged Smad expression constructs. Smad2, Smad3, and Smad4 each were detected in anti-E7 immunoprecipitates by protein immunoblotting with antibody to FLAG-tag for Smad2 and Smad3 and Myc-tag for Smad4 (Fig. 3, A–C). We found that E7 interacts with Smad2, Smad3, and Smad4 in a ligand-independent manner in vivo. The interaction between these Smad proteins and E7 was also studied by GST pull-down assays in vitro using 35S-labeled Smad2, -3, and -4 proteins. E7 interacted with 35S-labeled Smad2, -3, or -4 (Fig. 3D). These results demonstrated E7 binds to Smad2, -3, or -4 directly. To see whether phosphorylated Smad proteins are associated with E7, we transfected E7 and Myc-Smad3 constructs into HepG2 cells and were incubated with TGF-β. Interaction of phosphorylated Smad3 with E7 was tested by co-immunoprecipitation of Smad3 and E7, followed by the immunoblotting with anti-phospho Smad3 antibody. As shown in Fig. 4, E7 interacts with both phosphorylated and unphosphorylated forms of Smad3.

To determine the extent to which E7 might co-localize with Smads in vivo, we performed confocal microscopic analysis using anti-E7, anti-Smad2, anti-Smad3, and anti-Smad4 antibodies in HepG2 cells. E7 exhibited a characteristic distribu-
tion that was nearly uniform in the nucleus (Fig. 5, A–C). Staining for endogenous Smad proteins in E7-expressing cells revealed extensive co-localization of Smads with E7 in the nucleus, indicated by the yellow color on the overlapping panels. In the absence of TGF-β1 treatment, most endogenous Smad3 is localized in the cytoplasm (Fig. 5 B). However, regardless of TGF-β1 treatment, Smad3 was predominantly localized in the nucleus when Smad3 and E7 were co-transfected in HepG2 cells, and confocal microscopy revealed extensive co-localization of Smad3 with E7 (Fig. 5, B and D). Smad2 and Smad4 were also sequestered in the nucleus when co-transfected with E7, regardless of TGF-β1 treatment (Fig. 5 D). These results suggest that E7 may facilitate the nuclear translocation of Smad proteins in a ligand-independent manner.

E7 Blocks Smad3 Binding to the Smad3 Binding Site, CAGA—To test whether the interaction of E7 with Smads might inhibit the ability of Smads to bind DNA, a DNA-affinity precipitation assay with biotinylated oligonucleotides containing the Smad-binding element (CAGA box) (20) was performed. Using nuclear extracts isolated from E7-expressing Mv1Lu cells (Mv1Lu-LXSN-E7) and control cells (Mv1Lu-LXSN), with or without TGF-β1 treatment, we isolated active Smad3 using biotinylated DNA containing TGF-β1-responsive CAGA sequence. The amount of Smad3 bound to the CAGA sequence was markedly increased in nuclear extracts from control cells after treatment with TGF-β1 (Fig. 6 A). However, the amount of Smad3 bound to the CAGA sequence was markedly reduced in nuclear extracts from Mv1Lu cells expressing E7, even after TGF-β1 treatment (Fig. 6 A). To examine whether E7 inhibits the formation of the Smad-containing complex, we also performed a gel shift assay using an oligonucleotide encompassing a TGF-β-responsive element in the PAI-1 promoter (−586 − 551). Nuclear extracts were prepared from the control and E7-expressing Mv1Lu cells after TGF-β1 treatment. TGF-β1 treatment markedly increased the formation of Smad-containing complex in control Mv1Lu cells, whereas expression of E7 in Mv1Lu-E7 cells

![Diagram](https://example.com/diagram.png)

**Fig. 3.** E7 interacts with the Smad2, -3, and -4. HepG2 cells were transfected with E7 and either FLAG-tagged Smad2, FLAG-tagged Smad3, or Myc-tagged Smad4 constructs. Cells were treated with TGF-β1 for 2 h. Cell extracts were subjected to immunoprecipitation using an anti-E7 or anti-FLAG antibody and Gamma-bind beads (Amersham Biosciences, Uppsala, Sweden), followed by immunoblotting with anti-FLAG, anti-myc, or anti-E7 antibody (A–C). The expression of E7 and Smads was monitored as indicated. The interaction between Smads and E7 was examined by GST pull-down assay in vitro. Bacterially expressed GST-E7 and GST alone were incubated with [35S]methionine-labeled Smad proteins (D). 25% of [35S]methionine-labeled Smad proteins used for the assay were applied as controls (Input).

**Fig. 4.** E7 interacts with both phosphorylated and unphosphorylated Smad3. HepG2 cells were transfected with E7 and Myc-tagged Smad3 constructs. Cells were treated with TGF-β1 for 2 h. Cell extracts were subjected to immunoprecipitation using an anti-E7 antibody and Gamma-bind beads (Amersham Biosciences, Uppsala, Sweden), followed by immunoblotting with anti-myc or anti-phospho-Smad3 antibody. The expression of E7 and Smad3 was monitored as indicated.
prevented the formation of the Smad-containing complex (Fig. 6B). These data strongly indicate a direct inhibitory role of E7 on the formation of Smad-containing complex.

To examine the mechanism by which E7 inhibits binding of Smad3 to CAGA, we determined the effect of a transformation deficient mutant, E7<sup>D21–24</sup> (13, 25). This mutant E7 protein has lost the ability to induce abnormal centrosome duplication (14). In luciferase assays using either the TGF-β-responsive 3TP lux reporter construct or p800-Luc, a fragment of the PAI-1 promoter (18), E7<sup>D21–24</sup> failed to repress TGF-β1-induced transcription (Fig. 7, A and B). We next examined whether E7<sup>D21–24</sup> differs from E7 in its ability to interact with Smad3. By co-immunoprecipitation with Smad3 we found that, unlike the wild-type E7, the E7<sup>D21–24</sup> mutant failed to interact with Smad3 (Fig. 8A). To test whether the E7<sup>D21–24</sup> mutant would also lack the ability to block the binding of Smad3 to its DNA binding site, we performed the CAGA binding assay using nuclear extracts isolated from the HepG2 cells transfected with either wild-type E7 expression construct or E7<sup>D21–24</sup> expression construct. As shown in Fig. 8B, TGF-β1 treatment enhanced Smad3 binding to CAGA element in control HepG2 cells (lane 2 of upper panel). In HepG2 cells expressing wild-type E7, virtually no Smad3 was bound to the CAGA element (lanes 3 and 4). In contrast, Smad3 retained the ability to bind to the CAGA element in nuclear extracts isolated from the HepG2 cells transfected with the E7<sup>D21–24</sup> mutant (lane 5). These results suggest that the region of E7 between residues 21 and 24 is required for interaction of E7 with Smad3, as well as for E7 to repress TGF-β1-mediated transcription.

E7 Interacts with the MH1 Domain of Smad3—Because E7 inhibits Smad3 binding to its target DNA sequence, we wondered whether E7 interacts with the DNA binding domain of Smad3 protein. To determine the domain of Smad3 responsible for interaction with E7 in vivo, we performed immunoprecipitation assays using various FLAG-tagged Smad3 expression constructs along with a wild-type E7 construct. E7 was found to co-immunoprecipitate with full-length Smad3, as well as with the N-terminal MH1 domain. E7 did not immunoprecipitate with the C-terminal MH2 domain or middle linker domains of Smad3 (Fig. 9), demonstrating that the MH1 domain of Smad3 contains the E7 interaction domain.

We next examined whether E7 inhibits the complex formation between Smad3 and Smad4. After transfection of Smad3 and Smad4 constructs with or without E7 into HepG2 cells, we examined whether E7 blocks the complex formation between Smad3 and Smad4 by examining the level of Smad4 bound Smad3. TGF-β1 treatment induced interaction between Smad4 and Smad3 (Fig. 10, lane 3), whereas E7 expression almost completely blocked interaction between Smad3 and Smad4 (Fig. 10, lane 5).

DISCUSSION

TGF-β is an extracellular signaling molecule that activates a tumor suppressor pathway (4). The ability of TGF-β signaling to suppress tumor formation is linked to its ability to reversibly
arrest cell proliferation in the G1 phase of the cell cycle (5, 26–28). As cells transform into cancer, they commonly lose the ability to arrest cell cycle progression in response to TGF-β/H9252.

Interestingly, many of the changes in gene expression that are associated with transformation of normal cells into cancer also appear to block the ability of TGF-β/H9252 to arrest cell cycle progression (29, 30). Thus, many lines of evidence indicate that the functions of TGF-β/H9252 depend on the ability of TGF-β signaling to inactivate proto-oncogenes and activate tumor suppressor genes.

Using an alternative approach, studies revealing the evolutionarily conserved functions of the DNA tumor-inducing viruses have similarly led to the discovery and characterization of many key cellular proto-oncogenes and tumor suppressor genes. For example, adenovirus and the human papilloma virus structurally encode very divergent oncogenes, yet the oncogenes encoded by these two viruses have many functions in common that have survived thousands of years of evolutionary pressure. Cells transformed by stable transfection of DNA tumor virus oncogenes, including adenovirus E1A and HPV-16 E7 were found to be insensitive to TGF-β-induced c-myc repression and growth inhibition (28). Because pRb binding mutants of these oncogenes did not block TGF-β repression of myc/CAT expression, it was suggested that pRb is a key cellular factor involved in a pathway mediating TGF-β inhibitory effects on keratinocytes, and that inactivation of pRb might account for the loss of TGF-β responsiveness.

Recently, it was shown that TGF-β1 inhibits topo IIα/β expression in Mv1Lu cells (31). The authors suggested that resistance to TGF-β1-induced inhibition of topo IIα expression by HBV 16 E7 might be mediated primarily through the inactivation of pRb, however, the effects of E7 on the Smads that mediate TGF-β1 signaling were not directly investigated (14).
Smad proteins play a key role in the intracellular signaling of TGF-β. Upon TGF-β receptor activation, Smad2 and Smad3 become phosphorylated and form heteromeric complexes with Smad4. These complexes translocate to the nucleus where they control expression of target genes. In the present study, we have shown that E7 blocks TGF-β signaling by blocking binding of the Smad complex to its target DNA sequence. E7 binds to Smad2, Smad3, and Smad4, and blocks binding of Smad3 to its DNA binding site, CAGA (Fig. 5). The pRb binding-deficient HPV-16 E7 mutant E7ΔD21–24 showed a greatly diminished ability to bind to Smad3 compared with wild type. FLAG-tagged full-length and truncated Smad3 proteins were co-transfected into HepG2 cells together with E7 and isolated by immunoprecipitation with anti-FLAG antibody. The Smad3-bound E7 was detected by protein immunoblotting with an anti-E7 antibody (top). Cell lysates were blotted with anti-FLAG to confirm expression of FLAG-Smad3 (middle). The expression of E7 protein and E7 mutant ΔD21–24 in the lysates was detected using anti-E7 antibody (bottom). E7 sequesters Smad proteins in the nucleus, even in the absence of TGF-β signaling. Because of the importance of the data reported here, additional studies are underway to determine whether the Smad proteins that are associated with E7 in the nucleus are phosphorylated and whether the Smad proteins associate with E7 as part of a higher order complex.

Direct DNA-binding activity of Smad complexes has been shown to be mediated by N-terminal Smad domain (MH1) (32).

![Fig. 8. E7 mutant ΔD21–24 does not bind to Smad3 and fail to inhibit binding of the Smad3 to its binding site, CAGA.](image1)

| E7 | E7ΔD21–24 |
|----|-----------|
| Flag-Smad 3 | + | + | + |
| E7 or E7ΔD21–24 bound to Smad3 | + | + | + |

![Fig. 9. The MH1 domain of Smad3 is responsible for binding to E7.](image2)

A, schematic drawings of Smad3 truncation mutants. B, FLAG-tagged Smad3 proteins were co-transfected into HepG2 cells together with E7 and isolated by immunoprecipitation with anti-FLAG antibody. The expression of E7 protein and E7 mutant ΔD21–24 in the lysates was detected using anti-E7 antibody (bottom). The MH1 domain of Smad3 is responsible for binding to E7. A, schematic drawings of Smad3 truncation mutants. B, FLAG-tagged Smad3 proteins were co-transfected into HepG2 cells together with E7 and isolated by immunoprecipitation with anti-FLAG antibody. The expression of E7 protein and E7 mutant ΔD21–24 in the lysates was detected using anti-E7 antibody (bottom).

![Fig. 10. E7 blocks Smad3-Smad4 interaction.](image3)

Flag-Smad3 | + | + | + | + | + |
Myc-Smad4 | - | - | + | + | + |
E7 | - | - | + | + | + |
TGF-β1 | - | - | - | - | + |
Smad4 bound Smad3 | + | + | + | + | + |
Smad4 + E7 | + | + | + | + | + |
Myc + Smad3 Flag | - | + | + | + | + |

![Fig. 11. E7 blocks Smad3-Myc-Smad4 interaction.](image4)
HPV E7 Represses Smad-mediated TGF-β Signaling

Because our study shows that the N-terminal Mad homology 1 (MH1) region of Smad3 is required for the interaction between E7 and Smad3, it is likely that E7 blocks the interaction of Smad3 with its target sequence by binding to the MH1 domain of Smad3.

TGF-β is known to down-regulate the synthesis of Cdk4 and to inhibit the activity of cyclin E-Cdk2 in a pathway leading to a G1 arrest. Cdk4 and Cdk2 have been implicated as the G1 kinases responsible for phosphorylating and inactivating pRb in mid to late G1. TGF-β causes the accumulation of unphosphorylated pRb in response cells through the suppression of Cdk4 synthesis and Cdk2 activity (9, 24). In Mv1Lu cells expressing HPV 16 E7, TGF-β was unable to down-regulate the expression of Cdk4 and Cdk2, whereas a hyperphosphorylated form of pRb was increased even in the presence of TGF-β. Our results suggest that suppression of Smad activity by E7 may also contribute to the accumulation of hyperphosphorylated form of pRb.

Although the pathogenesis of cervical cancer is still not fully understood, substantial evidence indicates that the papillomavirus E7 protein is involved in the proliferation and transformation of normal human cervical cells (33, 34). The E7 protein is the major oncogenic protein produced by cervical cancer-associated human papillomaviruses (35). Our study is the first to demonstrate that HPV-positive cells develop resistance to the antiproliferative effects of TGF-β in part through the inhibition of Smad DNA binding activity by E7 oncoprotein. Thus, the ability to bind and inactivate Smad proteins appears to be a key function shared by the adenovirus E1A and HPV-16 E7 oncoproteins, which has been retained throughout years of evolution (32, 36). Our results suggest that inactivation of Smad-mediated signaling is necessary to block the tumor suppressor functions of TGF-β and that inactivation of pRb and p53 must be tightly coupled with the targeted inactivation of Smads for efficient transformation. By inactivating each of the most important brakes on their proliferation, HPV-infected cells can then multiply at high rates and show highly elevated TGF-β ligand and receptor expression (24). This considerable inhibition of TGF-β signaling by E7 thus provides a uniquely favorable environment for the development of cervical cancer.

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REFERENCES

1. Landis, S. H., Murray, T., Bolden, S., and Wingo, P. A. (1999) CA Cancer J. Clin. 49, 8–31

2. D. K. Lee, B.-C. Kim, I. Y. Kim, E.-a. Cho, D. J. Satterwhite, and S.-J. Kim, unpublished results.

3. Jones, D. L., Alani, R. M., and Münger, K. (1997) Genes Dev. 11, 2101–2111

4. Kim, S.-J., Im, Y.-H., Markowitz, S. D., and Bang, Y.-J. (2000) Cytokine Growth Factor Rev. 11, 159–168

5. Laiho, M., Decaprio, J. A., Ludlow, J. W., Livingston, D. M., and Massague, J. (1990) Cell 62, 175–185

6. Hannon, G. J., and Beach, D. (1994) Nature 371, 257–260

7. Pardali, K., Kurisaki, A., Moren, A., ten Dijke, P., Kardassis, D., and Moustakas, A. (2000) J. Biol. Chem. 275, 29244–29256

8. Reynisdottir, I., Poljak, K., Iavarone, A., and Massague, J. (1995) Genes Dev. 9, 1831–1845

9. Ewen, M. E., Shuss, H. K., Whitehouse, L. L., and Livingston, D. M. (1993) Cell 74, 1009–102010

10. Massague, J., and Wotton, D. (2000) EMBO J. 19, 1745–1754

11. Heldin, C.-H., Miyazono, K., and ten Dijke, P. (1997) Nature 390, 465–471

12. Massague, J. (1998) Annu. Rev. Biochem. 67, 753–791

13. Gonzalez, S. L., Stremlau, M., He, X., Basile, J. R., and Münger, K. (2001) J. Virol. 75, 7583–7591

14. Satterwhite, D. J., White, R. L., Matsunami, N., and Neufeld, K. L. (2000) Cancer Res. 60, 6989–6994

15. Kim, S.-J., Winokur, T. S., Lee, H.-D., Danielpour, D., Kim, K. Y., Geiser, A. G., Chen, L.-S., Sporn, M. B., Roberts, A. B., and Jay, G. (1991) Mol. Biol. Cell. 2, 5222–5228

16. Wrana, J. L., Attisano, L., Carcamo, J., Zentella, A., Doody, J., Laiho, M., Wang, X.-F., and Massague, J. (1992) Cell 71, 1003–1014

17. Zawel, L., Imai, J. L., Buckhaults, P., Zhou, S., Kinzler, K. W., Vogelstein, B., and Kern, S. E. (1998) Mol. Cell 1, 611–617

18. Denlinger, S., Itoh, S., Vivien, D., ten Dijke, P., Huet, S., and Gauthier, J.-M. (1998) EMBO J. 17, 3091–3100

19. Havir, I., Shamay, M., Doitsch, G., and Shaull, Y. (1998) Mol. Cell. Biol. 18, 1562–1569

20. Tada, K., Inoue, H., Ebisawa, T., Makuchii, M., Kawabata, M., Imamura, T., and Miyazono, K. (1999) Genes Cells 4, 731–741

21. Park, S. H., Lee, S. R., Kim, B. C., Cho, E. A., Patel, S. P., Kang, H.-B., Sauvage, E. A., Nakazishi, O., Trepeel, J. B., Lee, B. I., and Kim, S.-J. (2002) J. Biol. Chem. 277, 5168–5174

22. Satterwhite, D. J., Aakre, M. E., Gorska, A. E., and Moses, H. L. (1994) Cell Growth & Differ. 5, 789–799

23. Tsubari, M., Taipale, J., Tiihonen, E., Keski-Oja, J., and Laiho, M. (1999) Mol. Cell. Biol. 19, 3654–3663

24. Farley, J., Gray, K., Nycum, L., Prentice, M., Birrer, M. J., and Jakowlew, S. B. (1999) Gynecol. Oncol. 78, 113–122

25. Duensing, S., Lee, L. Y., Duensing, A., Basile, J., Poonsoniyom, S., Gonzalez, S., Crum, C. P., and Münger, K. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 10002–10007

26. Sporn, M. B., Roberts, A. B. (1992) J. Cell Biol. 119, 1017–1021

27. Howe, P. H., Draetta, G., and Leof, E. B. (1991) Mol. Cell. Biol. 11, 1185–1194

28. Pietenpol, J. A., Stein, R. W., Moran, E., Yaciuk, P., Schlegel, R., Lyons, R. M., Pittelkow, M. R., Munger, K., Howley, P. M., and Moses, H. L. (1990) Cell 61, 777–785

29. Massague, J., and Chen, Y. G. (2000) Genes Dev. 14, 627–644

30. Blobe, G. C., Schiemann, W. P., and Lodish, H. F. (2000) N. Engl. J. Med. 342, 1350–1358

31. Satterwhite, D. J., Matsunami, N., and White, R. L. (2000) Biochem. Biophys. Res. Commun. 276, 686–692

32. Feng, X. H., Zhang, Y., Wu, R. Y., and Derynck, R. (1998) Genes Dev. 12, 2153–2163

33. Pei, Y.-F., Sherman, L., Sun, Y. H., and Schlegel, R. (1998) Carcinogenesis 19, 1481–1486

34. Demers, G. W., Halbert, C. L., and Galloway, D. A. (1994) Virology 198, 169–174

35. Enzenauer, C., Mengue, G., Lavigne, A., Davidson, I., Pfister, H., and May, M. (1998) Intern. J. Cancer 41, 80–90

36. Nishihiara, A., Hanai, J., Imamura, T., Miyazono, K., and Kawabata, M. (1999) J. Biol. Chem. 274, 28716–28723
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