The Human Papillomavirus (HPV) 16 E2 Protein Induces Apoptosis in the Absence of Other HPV Proteins and via a p53-dependent Pathway*

Kenneth Webster‡, Joanna Parish§, Maya Pandya¶, Peter L. Stern‖, Anthony R. Clarke, and Kevin Gaston**

From the Department of Biochemistry, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, United Kingdom and the ‖Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Manchester M20 9BX, United Kingdom

The human papillomavirus (HPV) E2 protein regulates viral gene expression and is also required for viral replication. HPV-transformed cells often contain chromosomally integrated copies of the HPV genome in which the viral E2 gene is disrupted. We have shown previously that re-expression of the HPV 16 E2 protein in HPV 16-transformed cells results in cell death via apoptosis. Here we show that the HPV 16 E2 protein can induce apoptosis in both HPV-transformed and non-HPV-transformed cell lines. E2-induced apoptosis is abrogated by a trans-dominant negative mutant of p53 or by overexpression of the HPV 16 E6 protein, but is increased by overexpression of wild-type p53. We show that mutations that block the DNA binding activity of E2 do not impair the ability of this protein to induce apoptosis. In contrast, removal of both N-terminal domains from the E2 dimer completely blocks E2-induced cell death. Heterodimers formed between wild-type E2 and N-terminally deleted E2 proteins also fail to induce cell death. Our data suggest that neither the DNA binding activity of E2 nor other HPV proteins are required for the induction of apoptosis by E2 and that E2-induced cell death occurs via a p53-dependent pathway.

Papillomaviruses infect epithelial cells and generally induce the formation of benign hyperproliferative lesions. However, some papillomavirus types are associated with cancer. For example, human papillomavirus (HPV)[1] types 16 and 18 have been linked to cervical cancer in women (1) and bovine papillomavirus (BPV) types 2 and 4 have been linked to bladder cancer and cancer of the upper alimentary canal respectively, in cattle (2, 3). Human cervical cancers express the viral E6 and E7 oncogenes, and the products of these genes increase cell

proliferation and promote cell immortalization (for a review, see Ref. 4). The human papillomavirus E2 gene, or lack thereof, is also thought to play a major role in the development of cervical cancer. Most cervical cancers contain chromosomally integrated copies of the HPV genome in which the viral E2 gene has been disrupted (5). Furthermore, mutations in the E2 gene increase the immortalization capacity of HPV 16 (6).

The papillomavirus E2 genes encode sequence-specific DNA-binding proteins that regulate viral gene expression and are also required for viral DNA replication (reviewed in Ref. 7). The E2 proteins bind as dimers to multiple copies of an inverted repeat sequence found within the viral long control region. Depending on the particular virus and the particular E2 protein being studied, the binding of E2 to these sites can either activate or repress transcription of the E6 and E7 oncogenes. For example, the HPV 16 E2 protein activates transcription from the P97 promoter located at the 3′ end of the HPV 16 long control region, whereas, under exactly the same conditions, the BPV1 E2 protein represses P97 promoter activity (8, 9). Each subunit of the E2 dimer contains two domains separated by a flexible hinge: the N-terminal domain of each subunit mediates the regulation of transcription, whereas the C-terminal domain mediates DNA binding and dimerization (10). In bovine papillomaviruses, truncated E2 proteins that lack the N-terminal transcriptional domain are also expressed. These truncated E2 proteins (E2-TR) can repress transcription and can also form transcriptionally inactive heterodimers with full-length E2 (11).

The E2 proteins from HPV 16, HPV 18, and BPV1 all have dramatic effects on the proliferation and survival of cervical carcinoma cell lines. We have shown that expression of the HPV 16 E2 protein in SiHa cells, an HPV 16-transformed cell line that contains a single disrupted copy of the E2 gene, induces cell death via apoptosis (12). Similarly, the HPV 18 E2 protein induces apoptosis in HeLa cells, an HPV 18-transformed cell line that also contains disrupted copies of the E2 gene (13). Expression of the BPV1 E2 protein in either SiHa or HeLa cells has been shown to suppress proliferation, in part at least, by blocking the transition from G_1 to S phase (14–16). Because the proliferation assays used in these experiments score colony formation after several days in culture, BPV1 E2 might also induce apoptosis in these cell lines. There is also some evidence to suggest that the E2 proteins might have effects on non-HPV-transformed cells. Expression of the HPV 31 E2 protein in HPV-negative normal human foreskin keratinocytes (NHK cells) using a recombinant adenovirus resulted in S phase cell cycle arrest and the appearance of cells with sub-G_0 DNA content; a characteristic feature of apoptotic cell death (17). However, BPV1 E2 has no effect on the proliferation
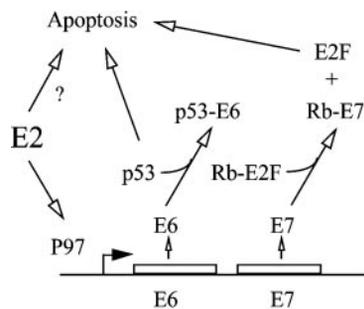
FIG. 1. **E2 regulates E6 and E7 gene expression.** A schematic representation of some of the possible routes from the HPV 16 E2 protein to the induction of apoptosis. The *bottom line* represents the integrated HPV genome, and the *bent arrow* indicates the P97 promoter. The E2 protein regulates transcription of the HPV 16 E6 and E7 genes (*open boxes*). E6 binds to p53 and this reduces the half-life of p53 within the cell. E7 binds to Rb and brings about the release of E2F. Both p53 and E2F can bring about apoptosis (see text for details). E2 could also induce apoptosis independently of its effects on transcription of E6 and E7.

of C33a cells, an HPV-negative cervical carcinoma cell line, or SAOS cells, an HPV-negative osteosarcoma cell line (15). Furthermore, the HPV 18 E2 protein has no effect on the levels of apoptosis in C33a cells, SAOS cells, or HaCat cells, an HPV-negative spontaneously immortalized human keratinocyte cell line (13).

At present, there is no model that can explain satisfactorily the effects of the E2 proteins on cell proliferation. BPV1 E2 and HPV 18 E2 have been shown to repress transcription of the HPV 18 E6 and E7 oncogenes (14, 13). The E6 protein binds to p53, and this interaction results in a decrease in the half-life of p53 within cells (18–21). Because p53 can block cell cycle progression and/or induce apoptosis (for a review, see Ref. 22), decreased levels of E6 might be expected to lead to increased levels of p53 and increased levels of cell cycle arrest and/or cell death (shown schematically in Fig. 1). In keeping with this view, expression of BPV1 E2 in HeLa cells appears to stabilize p53 (14, 13). However, E2-TR also represses transcription of E6 and E7 in these cells, but this truncated E2 protein can neither stabilize p53 nor induce apoptosis (13). In addition, the expression of HPV 31 E2 in NHK cells appears to destabilize p53 (17).

The E7 protein binds to the Rb tumor suppressor protein and the Rb-related proteins p107 and p130 (23, 24). The binding of E7 to Rb brings about the release of E2F proteins from Rb-E2F complexes and is also thought to target Rb for ubiquitin-dependent proteolysis (25–27). When released from Rb, members of the E2F family of transcription factors activate the transcription of genes required for S phase, and overexpression of E2F-1 can induce apoptosis in serum-starved cells (28, 29). The repression of E7 transcription by E2 might therefore be expected to reduce the levels of free E2F, leading to cell cycle arrest (see Fig. 1). Expression of BPV1 E2 protein in HeLa cells is accompanied by decreased levels of E2F-1 mRNA and protein and by reduced expression of E2F-dependent genes (16). However, expression of the HPV 16 E2 protein in SiHa cells is accompanied by increased E2F activity (12). Furthermore, overexpression of the HPV 31 E2 protein in NHK cells is accompanied by an increase in E2F-1 mRNA levels (17). Another complication is that unlike BPV1 E2, which represses transcription, the HPV 16 and HPV 18 E2 proteins have both been shown to activate transcription of the HPV 16 E6 and E7 oncogenes (8, 9). Any increase in the levels of E7 might be expected to result in increased levels of free E2F and this could in turn lead to cell death (12).

Models that seek to explain the effects of E2 proteins on cell proliferation via transcriptional repression or activation of the

E6 and E7 genes are obviously limited to HPV-positive cells. However, the HPV 31 E2 protein appears to induce apoptosis in HPV-negative NHK cells (17). Moreover, mutations in the HPV 16 long control region that block the binding of E2 to the promoter proximal E2 sites and prevent E2-mediated repression of E6 and E7 transcription do not fully relieve the negative effects of E2 on transformation efficiency (6). These reports suggest that E2 might influence cell proliferation independently of its effects on the transcription of E6 and E7. To address this issue, we have expressed the HPV 16 E2 protein in a variety of cell lines. We show that this E2 protein can induce apoptosis in both HPV-transformed and non-HPV-transformed cell lines. In addition we show that the HPV 16 E2-induced apoptosis is p53-dependent and that the DNA binding activity of this E2 protein is not required for the induction of cell death.

EXPERIMENTAL PROCEDURES

Plasmids—The plasmids pCB6+p53 and pCB6+p53173L express wild-type and mutant p53, respectively, and were kindly supplied by Drs. Moshe Oren and Andy Phillips. Plasmid pCMX-GFP3 expresses green fluorescent protein (GFP) and was kindly supplied by Dr. Jeremy Tavaré. The pWEB plasmid was made by removing an *Xho*I-*Eco*RI fragment carrying the cytomegalovirus promoter from pUHD 15–1 and using this fragment to replace the tetracycline-inducible promoter in pUHD 10–3. The HPV 16 E2, E6, and E7 expression plasmids were produced by cloning the appropriate HPV sequences into a unique *Eco*RI site in pWEB, immediately downstream of the cytomegalovirus promoter.

The E2 gene was amplified by PCR (94 °C for 1 min, 53 °C for 3 min, and 72 °C for 1 min, for 30 cycles) from HPV 16 DNA template using the oligonucleotide primers E2$_{5'}$ (5′-CTAC**GAATTC**ATGGAGACTCTTTG-CCAACG-3′) and E2$_{3'}$ (5′-GATA**GAATTC**TCATATAGACATAAATCC-AG-3′). These primers place *Eco*RI restriction sites (highlighted in boldface) at the 5′ and 3′ ends of the E2 coding sequence. The PCR product was cloned into the *Eco*RI site of pWEB and sequenced using a panel of E2-specific sequencing primers to check for the occurrence of any point mutations.

The E6 gene was amplified by PCR (95 °C for 1 min, 52 °C for 1 min, and 68 °C for 2 min, for 30 cycles) from HPV 16 template using the primers E6$_{5'}$ (5′-TGA**GAATTC**ATGCACCAAAAGAGAACTGCAATGT-TTCAG-3′) and E6$_{3'}$ (5′-ATC**GAATTC**TTACAGCTGGGTTTCTCTAC-G-3′). The PCR product was cloned into the *Eco*RI site of pWEB and sequenced using a panel of E6-specific sequencing primers.

The E7 gene was amplified by PCR (94 °C for 1 min, 54 °C for 2 min, and 72 °C for 1 min, for 30 cycles) from HPV 16 template using the primers E7$_{5'}$ (5′-TCG**GAATTC**ATGCATGGAGATACACCTAC-3′) and E7$_{3'}$ (5′-AGC**GAATTC**TTATGGTTTCTGAGAACAGATGG-3′). The PCR product was cloned into pWEB and sequenced using the E7 PCR primers.

Mutated E2 constructs were generated using PCR-directed mutagenesis. The plasmid pWEB-E2$_{DBDm}$ expresses a mutated E2 protein in which three amino acids within the E2 DNA binding domain (Asn-296, Lys-299, and Arg-304) have been replaced by alanines. The mutations were introduced by PCR (94 °C for 1 min, 55 °C for 1 min, and 68 °C for 1 min, for 30 cycles) using the primers pWEB$_{5'}$ (5′-ACCTCCATAGAA-GACACCGGG-3′) and E2 m (5′-CGACA**CTGCAG**TATACAATGTACA-ATGCTTTTTAAA*TGC*ATATCTTAAACA*TGC*TAAAGT*AGC*AGCATC-ACC-3′) with pWEB-E2 as template. The bases in italics mismatch the E2 gene and introduce a *Pst*I site (highlighted in boldface) at its 3′ end. This site and an *Sst*I site located within the cytomegalovirus promoter were used to replace the wild-type E2 sequence in pWEB-E2 with the mutated E2$_{DBDm}$ sequence. The entire PCR product was sequenced using a panel of E2-specific sequencing primers to check for the occurrence of any unwanted mutations.

The plasmid pWEB-E2Ct expresses a truncated E2 protein that lacks the N-terminal amino acids of E2 from 1 to 279 but dimerizes and binds DNA normally.[2] To create this mutant, HPV 16 sequences between base pairs 3592 and 3852 were amplified by PCR (94 °C for 1 min, 55 °C for 1 min, and 68 °C for 1 min, for 30 cycles) using the primers E2$_{Ct5'}$ (5′-GAAACA**GAATTC***ATG*AACTGTAATAGTAACACTACACCC-3′) and E2$_{3'}$ with pWEB-E2 as template. These primers place *Eco*RI restriction sites (boldface) at both ends of the product and introduce a

---

[2] Lewis, H., and Gaston, K. (1999) *J. Mol. Biol.* **294,** 885–896

translation start codon (italics). The PCR product was cloned into the *Eco*RI site in pWEB and sequenced using E2-specific primers. The plasmid pWEB-E2Ct$_{DBDm}$ expresses a DNA binding-defective version of E2Ct. This plasmid was produced exactly as described for pWEB-E2Ct except that pWEB-E2$_{DBDm}$ was used as template in the PCR.

The 86-amino acid E2Ct and E2Ct$_{DBDm}$ proteins were expressed in *Escherichia coli* XL1-blue cells using the expression vector pKK223–3 (Amersham Pharmacia Biotech). The sequences encoding E2Ct and E2Ct$_{DBDm}$ were excised as *Eco*RI fragments from pWEB-E2Ct and pWEB-E2Ct$_{DBDm}$, respectively, and cloned into a unique *Eco*RI site downstream of the P$_{tac}$ promoter in pKK223–3. The inserts were sequenced using E2-specific and pKK223–3-specific primers.

Protein Purification and Circular Dichroism Spectroscopy—*E. coli* XL1-blue cells containing either pKK-E2Ct or pKK-E2Ct$_{DBDm}$ were grown to an $A_{600 nm}$ of 0.5. Protein expression was then induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside, and the cells incubated at 37 °C overnight. The cells were harvested by centrifugation, resuspended in 50 mM Tris-acetate-EDTA buffer (pH 7.5) containing 1 mM MgCl$_2$ and 1% 2-mercaptoethanol and then lysed by sonication at 4 °C. The cell lysate was cleared by centrifugation (15,000 × *g* for 30 min at 4 °C) and then incubated with 0·1% DNase I for 30 min at 20 °C. The cell extract was dialyzed for 3 h against 50 mM phosphate buffer (pH 5.7) containing 1% 2-mercaptoethanol and then recentrifuged. The supernatant was loaded onto an S-Sepharose cation exchange medium column equilibrated in 50 mM phosphate buffer (pH 5.7) containing 10 mM dithiothreitol. After washing with 50 column volumes of phosphate buffer, the E2 protein was eluted using a linear gradient of 0.2–1 M NaCl in the same buffer over 500 ml (at 1 ml/min). Protein peaks (detected by $A_{280 nm}$) were collected and analyzed by SDS-polyacrylamide gel electrophoresis and gel retardation assays (data not shown). Pooled E2 fractions were dialyzed against 10 volumes of 50 mM phosphate buffer (pH 5.7) containing 10 mM dithiothreitol for 3 h and then applied to a MonoS HR 16/10 cation exchange fast protein liquid chromatography column equilibrated in the same buffer. E2 was eluted with a 0.1–1 M NaCl gradient and dialyzed against 25 mM sodium phosphate buffer (pH 7.9) containing 1 mM dithiothreitol for 3 h before being snap frozen and stored at −70 °C. Isoelectric points (pI) and molecular weight values were determined from the amino acid sequences of wild-type (pI 9.7; M_r 10016.6) and mutant E2 (pI 9.4; M_r 9831.4) using Expasy Tools. Molecular weights were confirmed on a VG Quattro triple quadrupole mass spectrophotometer with electrospray ionization. Structural integrity was confirmed using far-UV and near-UV circular dichroism spectroscopy on a Jobin Yvon CD6 spectropolarimeter using a 0.05-cm path length with a 0.5-nm resolution at 1 nm/min.

Gel Retardation Assays—A double-stranded oligonucleotide (100 ng) corresponding to the sequence of the HPV 16 E2 site 1 from nucleotides 46 to 65 (5′-TTGAACCGAAACCGGTTAGT-3′) was end labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase. Unincorporated label was removed using a Sephadex G-50 column (Stratagene). Labeled oligonucleotides (10,000 cpm) were incubated with purified proteins in binding buffer (20 mM HEPES (pH 7.9), 25 mM KCl, 1 mM dithiothreitol, 0.1% Nonidet P-40, 10% glycerol, 0.5 μg/μl bovine serum albumin, 80 ng/μl poly[d(I·C)]). After 20 min at 20 °C, free and bound labeled DNA were resolved on 6% nondenaturing polyacrylamide gels run in 0.5× TBE and visualized by autoradiography. Heterodimers between wild-type E2Ct and E2Ct$_{DBDm}$ were formed by mixing and denaturing the proteins in 3 M urea (1 h at 20 °C) and then refolding by dilution to 0.1 M urea in binding buffer. The DNA binding activity of the heterodimers was assayed exactly as described above.

Cell Culture and Transfections—SiHa, C33a, and COS-7 cells were maintained in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal bovine serum (Sigma) and penicillin (100 000 units/liter) and streptomycin (100 mg/liter). NIH 3T3 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% calf serum (Sigma) and penicillin/streptomycin. HeLa cells were maintained in minimal essential medium (Sigma) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and penicillin/streptomycin. 866, 873, 877, 915, and 808F cells were maintained in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum, penicillin/streptomycin, 2 mM L-glutamine, 5 μg/ml insulin, 0.01 μg/ml epidermal growth factor, 0.01 μg/ml cholera toxin, and 0.4 μg/ml hydrocortisone. All cells were maintained at 37 °C in 5% CO$_2$.

Prior to transient transfection, cells were seeded at 3×10^5 cells/well onto coverslips in six-well plates and incubated overnight to obtain a subconfluent culture. The liposome-based reagents Tfx-50 (for SiHa and NIH3T3 cells) and Tfx-20 (for all other cell lines) (Promega) were used at a 3:1 liposome:DNA ratio in 1 ml of serum-free media per transfection, according to the manufacturer's instructions. The DNAs used in
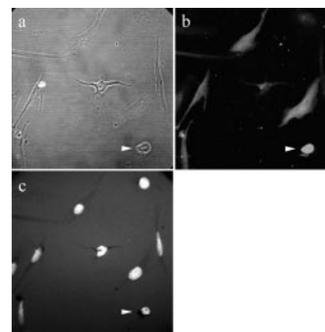

FIG. 2. **E2 induces chromatin condensation and membrane blebbing in HeLa cells.** A representative group of HeLa cells visualized using a × 40 oil immersion lens fitted to an epifluorescent microscope: *a,* bright field microscopy; *b,* GFP fluorescence (transfected cells were identified using an fluorescein isothiocyanate filter set); *c,* 4′,6′-diamidino-2-phenylindole fluorescence. Chromatin was visualized using bisbenzimide (Hoechst stain) and a 4′,6′-diamidino-2-phenylindole filter set. In each panel, the same apoptotic cell is indicated by the *arrowhead.* Membrane blebbing is seen in *a* and *b.* Chromatin condensation is seen in *c.*

each transfection are described in the text above. After 18 h (E7 experiments) or 30 h (E2 experiments), the coverslips were washed in phosphate-buffered saline, and the cells were fixed with 4% paraformaldehyde/phosphate-buffered saline at 22 °C for 30 min. Following further washes with phosphate-buffered saline, the cells were stained with bisbenzimide (Hoechst no. 33258, Sigma) for 30 min before being washed in phosphate-buffered saline and mounted onto microscope slides in 10 μl of MOWIOL (Calbiochem).

Fluorescence Microscopy and Imaging—Fluorescence microscopy was carried out using a Leica DM IRBE inverted epifluorescent microscope fitted with fluorescein isothiocyanate and 4′,6′-diamidino-2-phenylindole filter sets and a × 20 air objective (Leica). Imaging was carried out using a Leica DM IRBE inverted confocal microscope using a × 40 oil objective (Leica) and TCS-NT4 software (Leica).

RESULTS

In our previous work, we looked at the effects of E2 expression in SiHa cells using stable cell lines that carry the HPV 16 E2 gene under the control of the heavy metal-inducible metallothionein promoter (12). The induction of E2 expression in these cells resulted in reduced cell proliferation and increased levels of cell death. The E2-induced cell death showed several of the features characteristic of apoptosis including: blebbing of the plasma membrane, chromatin condensation, and the appearance of cell fragments with sub-G$_0$ DNA content (12). Using this approach to look at the effects of E2 expression in a variety of cell lines would require the production of numerous stable cell lines. Because we know that the E2 protein can induce apoptosis in at least some cell lines, this would be a time-consuming and difficult task. In addition, to extend our work we also wanted to look at the effects of expressing E6 and E7, either individually or in conjunction with E2. To this end, we have used transient transfection to express the HPV 16 E2, E6, and E7 proteins in different cell lines.

The HPV 16 E2 and E7 Proteins Induce Apoptosis in HeLa Cells—The plasmids pWEB-E2, pWEB-E6, and pWEB-E7 express the HPV 16 E2, E6, and E7 proteins, respectively. Each of these plasmids was transiently transfected into HeLa cells growing on coverslips using liposomes (Fig. 2). In each experiment, the plasmid pCMX-GFP3 was co-transfected into the cells; pCMX-GFP3 expresses the GFP and allows transfected cells to be identified by their fluorescence upon excitation through a fluorescein isothiocyanate filter set. Because GFP is expressed uniformly throughout the transfected cell, it also allows the assessment of cellular morphology (Fig. 2*b*). The cells were stained with bisbenzimide (Hoechst stain), which enters the nuclei of all of the cells present, regardless of their transfection status, and allows a comparison of chromatin con-
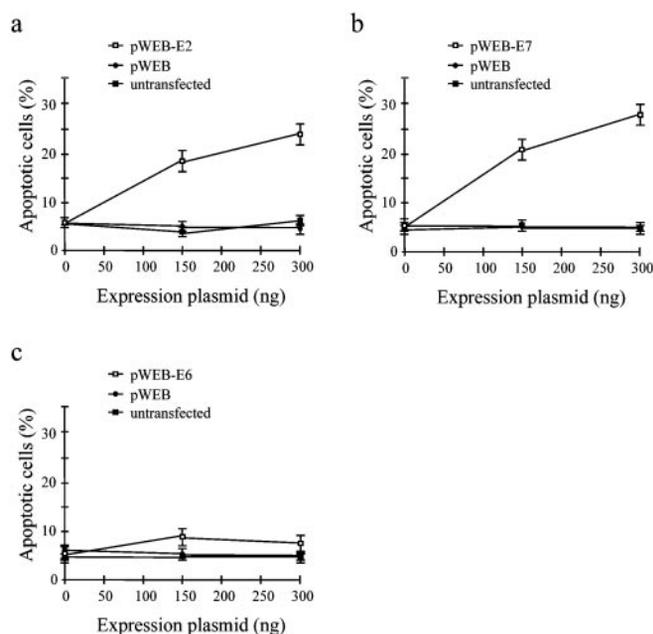
The HPV 16 E2 Protein Induces p53-dependent Apoptosis

FIG. 3. **E2 and E7 but not E6 induce apoptosis in HeLa cells.** *a,* the GFP-expressing plasmid pCMX-GFP3 and increasing amounts of either pWEB or pWEB-E2 were transiently transfected into HeLa cells. Apoptotic cells in the transfected and untransfected populations were identified as shown in Fig. 2. The transfection was performed in duplicate and repeated at least three times. In *b* and *c,* the experiment shown in *a* was repeated using pWEB-E7 and pWEB-E6, respectively, in place of pWEB-E2.

densation between untransfected cells and transfected cells within the population (Fig. 2*c*). Individual cells were scored as untransfected or transfected using GFP and then assessed for chromatin condensation and membrane blebbing using Hoechst stain and GFP, respectively. A typical transfected cell that is undergoing apoptosis is indicated in Fig. 2. The percentage of untransfected cells and transfected cells undergoing apoptosis was determined by counting. At least 100 untransfected cells and 100 transfected cells were counted, and each experiment was repeated a minimum of three times. Although this method is laborious, it is more reliable than other methods used to count apoptotic cells and allows the assessment of individual cells.

The percentage of apoptotic HeLa cells seen after transient transfection with the E2-, E6-, and E7-expressing plasmids is shown in Fig. 3. In each experiment, around 5% of the untransfected cells and around 5% of the cells transfected with the empty pWEB vector are apoptotic. Both the E2 and the E7 expression plasmids produced a significant increase in the levels of apoptosis within the transfected population (Fig. 3, *a* and *b,* respectively). In contrast, the E6 expression plasmid had little or no effect (Fig. 3*c*). The amount of plasmid required for the maximum induction of cell death and the time at which maximum death is observed differ between the E2- and E7-expressing plasmids. E2-induced apoptosis occurs maximally after around 30 h and with 300 ng of expression plasmid, whereas E7-induced apoptosis occurs maximally after around 18 h and with 800 ng of expression plasmid (not shown). These data demonstrate that the HPV 16 E2 and E7 proteins both induce apoptosis in HeLa cells. We next set out to determine whether these proteins can induce apoptosis in other cell lines.

E2 and E7 Induce Apoptosis in Both HPV-transformed and Non-HPV-transformed Cell Lines—We assayed the ability of the HPV 16 E2 and E7 proteins to induce apoptosis in six HPV-transformed cell lines and four non-HPV-transformed cell lines. The results of this comparison are shown in Table I. In

each experiment, the transfection efficiency varied from 5 to 10% depending on the cell line. Both E2 and E7 induced high levels of apoptosis in HeLa cells and SiHa cells, which are HPV 18- and HPV 16-transformed cervical carcinoma cell lines, respectively. Both E2 and E7 also induced high levels of apoptosis in human 866, 873, 877, and 915 keratinocytes: 866 cells and 915 cells contain HPV 16, 873 cells contain HPV 18, and 877 cells contain both HPV 18 and HPV 45 (31).[3] Interestingly, both E2 and E7 failed to induce apoptosis in either C33a cells or COS-7 cells, a non-HPV-transformed cervical carcinoma cell line and an SV40-transformed monkey fibroblast cell line, respectively. However, E2 and E7 did induce high levels of apoptosis in two other HPV-negative cell lines: 808F cells and NIH3T3 cells, which are a human fibroblast cell line and a mouse fibroblast cell line, respectively. Thus, E2 is capable of inducing apoptosis in at least two HPV-negative cell lines. Another striking feature of these results is that all the cell lines induced to undergo apoptosis by E2 are also induced to undergo apoptosis by E7. Similarly, the cell lines that are not sensitive to E2 expression are not sensitive to E7 expression. These data suggest that E2 and E7 induce apoptotic cell death via the same pathway or via pathways that converge at some point.

All of the cell lines that were seen to undergo apoptosis in response to E2 or E7 expression are thought to contain wild-type p53. For example, NIH 3T3 cells contain wild-type p53 and can undergo p53-dependent apoptosis (32). In contrast, C33a cells contain mutated p53 (33), and these cells fail to undergo apoptosis in response to either E2 or E7. Although COS-7 cells contain wild-type p53, these cells express the SV40 T antigen, which has been shown previously to efficiently sequester p53 (34). To determine whether p53 plays a role in E2 and/or E7-induced cell death, we next looked at the effects of a trans-dominant negative p53 mutant and expression of the HPV 16 E6 protein on the levels of apoptosis in cells expressing these proteins.

E2 and E7 Induce Apoptosis via a p53-dependent Pathway— HeLa cells were transiently co-transfected with pWEB-E2 or pWEB-E7 and either pCB6+p53, which expresses wild-type p53, or pCB6+p53173L, which expresses a trans-dominant negative p53 mutant. Co-expression of wild-type p53 increased the levels of apoptosis induced by both E2 and E7 by almost 50% (Fig. 4*a,* compare *columns 4* and *5* to *columns 7* and *8*). In contrast, co-expression of the trans-dominant negative p53173L mutant decreased the level of apoptosis induced by both E2 and E7 to near the basal level (Fig. 4*a, columns 6* and *9,* respectively). These data suggest that the apoptosis induced by the HPV 16 E2 and E7 proteins occurs through a p53-dependent pathway. To confirm this conclusion, HeLa cells were transiently co-transfected with pWEB-E2 or pWEB-E7 and either pWEB-E6 or the empty pWEB vector. The HPV 16 E6 protein binds p53 in conjunction with the E3 ubiquitin ligase enzyme E6-AP, and this results in the degradation of p53 via a ubiquitin-dependent protease (19, 35). The addition of increasing amounts of the pWEB-E6 plasmid resulted in a gradual decrease in the level of E2-induced apoptosis (Fig. 4*b*). Similarly, the level of apoptosis induced by the E7 protein was also significantly reduced by the co-expression of E6 (Fig. 4*c*). In the presence of large amounts of pWEB-E6, the levels of both E2- and E7-induced apoptosis were reduced to around the basal level. Taken together, these results firmly establish that functional p53 is required for apoptosis induced by both the HPV 16 E2 protein and the HPV 16 E7 protein.

The DNA Binding Activity of E2 Is Not Required for the Induction of Apoptosis—Although there are several plausible

---

[3] P. Stern, unpublished observations.

TABLE I

E2 and E7 induce apoptosis in a variety of cell lines

Cell line	HPV status	Apoptotic cells[a]		
		Background[b]	HPV 16 E2	HPV 16 E7
		%		
HeLa	HPV 18	5 ± 2	26 ± 3	27 ± 3
SiHa	HPV 16	5 ± 2	44 ± 3	46 ± 3
866	HPV 16	5 ± 2	22 ± 2	22 ± 2
873	HPV 18	7 ± 2	35 ± 3	24 ± 2
877	HPV 18, 45	6 ± 2	28 ± 3	28 ± 3
915	HPV 16	7 ± 2	33 ± 3	23 ± 2
C33a		8 ± 2	9 ± 2	8 ± 3
COS-7		6 ± 2	6 ± 2	5 ± 2
808F		6 ± 2	26 ± 3	20 ± 3
NIH3T3		6 ± 2	25 ± 3	20 ± 2

[a] The percentage of apoptotic cells was determined as described in the text. The data are presented as the mean and S.D. of at least three separate experiments.
[b] The background level of apoptosis refers to the untransfected population and the population transfected with the empty pWEB plasmid.

FIG. 4. **E2- and E7-induced apoptosis is p53-dependent.** *a,* HeLa cells were transiently transfected with the GFP-expressing plasmid pCMX-GFP3; either pWEB (*columns 1–3*), pWEB-E2 (*columns 4–6*), or pWEB-E7 (*columns 7–9*); and either 200 ng of pCB6+p53, which expresses wild-type p53 (*wt*), or 200 ng of pCB6+p53173L, which expresses mutant p53 (*mt*). Apoptotic cells were identified as in Fig. 2. The transfection was performed in duplicate and repeated three times. *b,* 300 ng of pWEB-E2 and increasing amounts of pWEB-E6 were transiently transfected into HeLa cells. Details as in Fig. 3. *c,* 800 ng of pWEB-E7 and increasing amounts of pWEB-E6 were transiently transfected into HeLa cells. Details as in Fig. 3.
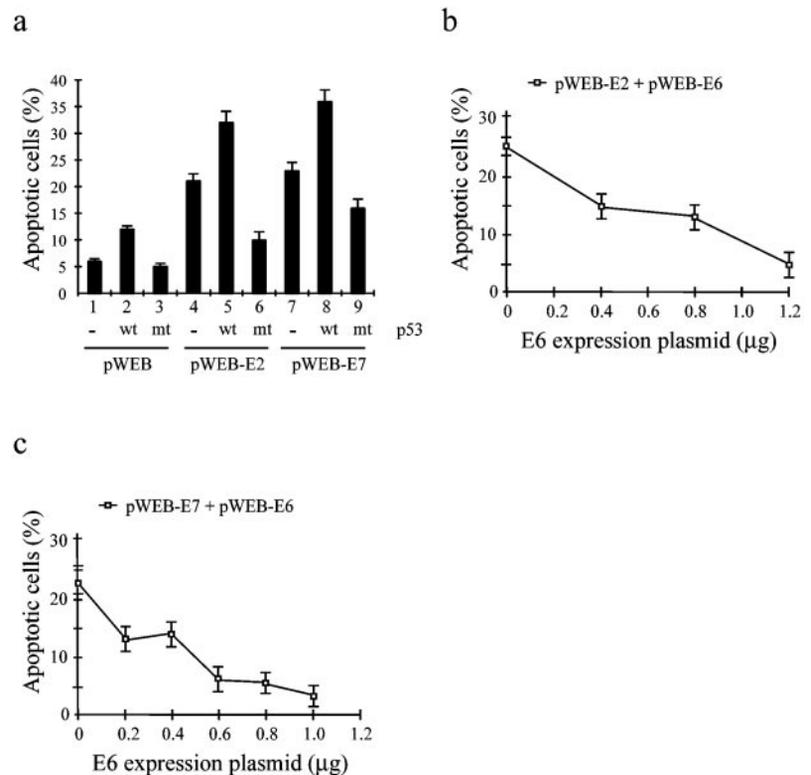
mechanisms whereby E7 overexpression could result in apoptosis, the route from E2 overexpression to apoptosis is unclear. We originally proposed that in SiHa cells, E2 might increase transcription of the integrated E7 oncogene and that this might result in E7-induced cell death. However, here we have shown that E2 can induce apoptosis in at least two non-HPV-transformed cell lines (Table I). These data imply that E2 does not kill cells simply by activating transcription of E7. To confirm this hypothesis we placed three point mutations within the E2 DNA binding domain (DBD) at positions known to be important for protein-DNA interactions. The crystal structures of the HPV 16 E2 DBD and the BPV1 E2 DBD-DNA complex suggest that amino acids Asn-296, Lys-299, and Arg-304 within the HPV 16 E2 DBD are critical for the recognition of specific E2 binding sites (36, 37). Using site-directed mutagenesis, we replaced all three of these amino acids with alanines. The mutations were introduced in the context of both the full-length E2 protein and the E2 DNA binding domain alone.

To establish that these mutations abolish DNA binding activity without disrupting the overall folding of the E2 DBD, we expressed both the wild-type DBD and the mutated DBD in bacteria. The plasmid pKK-E2Ct expresses a truncated E2 protein (amino acids 280–365) that can dimerize and bind DNA normally.[2] The plasmid pKK-E2Ct$_{DBDm}$ expresses the equivalent E2 fragment containing the N296A, K299A, and R304A mutations. The E2Ct and E2Ct$_{DBDm}$ proteins were purified from bacteria carrying the respective plasmids (Fig. 5a). Circular dichroism (CD) was then used to test whether the presence of the mutations altered the folding or dimerization of the E2 DBD. The CD spectra for the E2Ct and E2Ct$_{DBDm}$ proteins (Fig. 5, *b* and *c*) are very similar. This implies that the mutations have little or no effect on these properties. Fig. 5*d* shows the results of a gel retardation assay in which increasing amounts of the E2Ct protein (*lanes 2–4*) or the E2Ct$_{DBDm}$ protein (*lanes 5–7*) were added to labeled oligonucleotides carrying an E2 binding site. As can be seen from Fig. 5, E2Ct binds tightly to the labeled DNA, whereas E2Ct$_{DBDm}$ exhibits no detectable binding to this site.

To determine whether the DNA binding activity of E2 is required for the induction of cell death, we transiently trans-

FIG. 5. **E2Ct mutated at Asn-296, Lys-299, and Arg-304 folds and dimerizes but fails to bind DNA.** *a,* samples of purified E2Ct and E2Ct$_{DBDm}$ were analyzed by SDS-polyacrylamide gel electrophoresis. The sizes of the markers used are indicated at the left (in thousands). *b* and *c,* circular dichroism was used to determine whether the presence of the Asn-296, Lys-299, and Arg-304 mutations affected the folding or dimerization of E2Ct$_{DBDm}$. *d,* increasing amounts (10, 50, and 250 nM, respectively) of E2Ct (*lanes 2–4*) or E2Ct$_{DBDm}$ (*lanes 5–7*) were added to labeled oligonucleotides carrying E2 binding site 1 from the HPV 16 genome: E2(1). Free and bound DNA was separated on a 6% polyacrylamide gel and visualized by autoradiography. The E2Ct-E2(1) complex is indicated by an *arrowhead*.
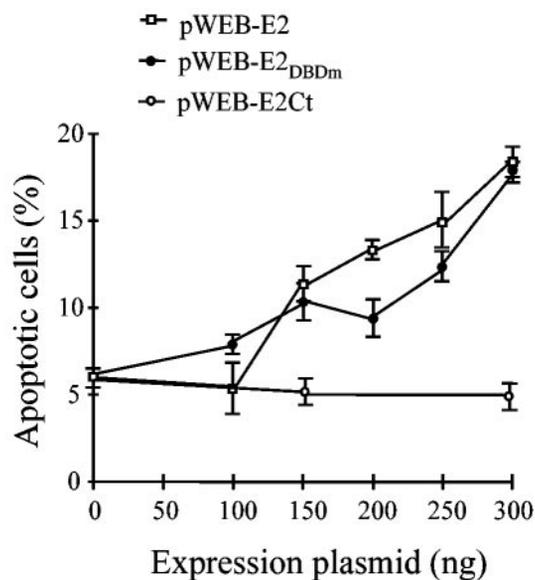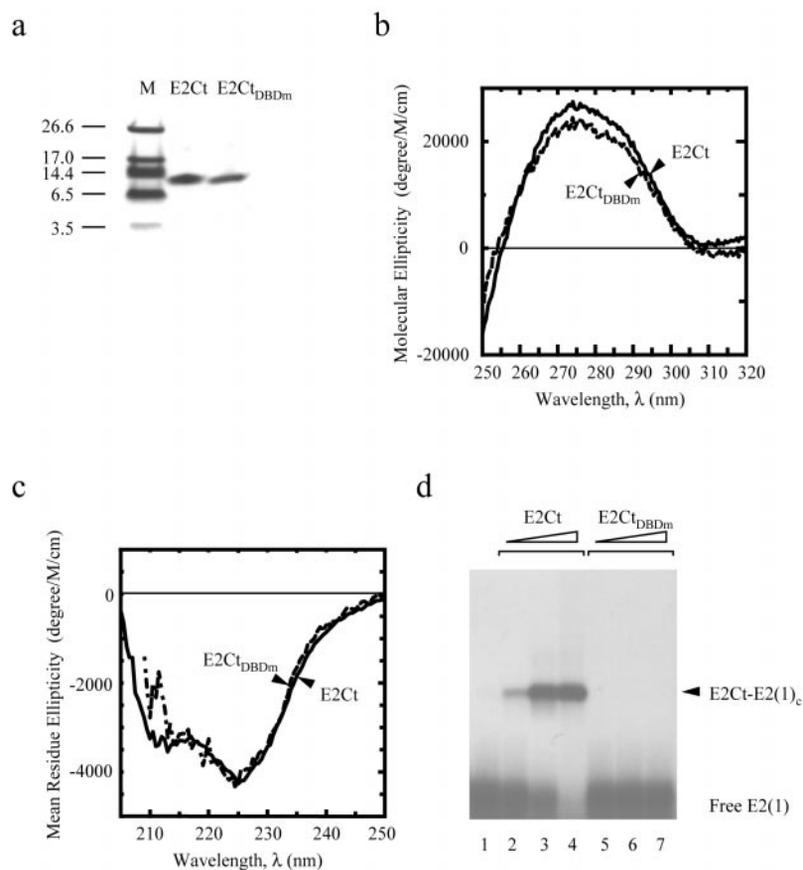
FIG. 6. **DNA binding is not required for the induction of apoptosis.** The GFP-expressing plasmid pCMX-GFP3 and increasing amounts of either pWEB-E2, pWEB-E2$_{DBDm}$, or pWEB-E2Ct were transiently transfected into HeLa cells. Apoptotic cells were identified as shown in Fig. 2. The transfection was performed in duplicate and repeated three times.

fected HeLa cells with plasmids expressing either the wild-type full-length E2 protein (pWEB-E2) or full-length E2 carrying the N296A, K299A, and R304A mutations (pWEB-E2$_{DBDm}$). Somewhat surprisingly, the pWEB-E2 and pWEB-E2$_{DBDm}$ plasmids induced almost identical levels of cell death (Fig. 6). In contrast, the plasmid pWEB-E2Ct, which expresses the E2 DBD alone and therefore lacks the N-terminal transcription activation domain, failed to induce cell death (Fig. 6). Thus,

although the sequence-specific DNA binding activity of E2 is not required for the induction of apoptosis in HeLa cells, the N-terminal transcription activation domain is indispensable. To confirm and extend these conclusions, we next looked at the ability of E2 heterodimers to induce cell death.

Two Functional N-terminal Domains Are Required for E2-induced Cell Death—The BPV1 E2 and E2-TR proteins have previously been shown to form heterodimers (11). Although these heterodimers are reported to bind DNA *in vitro*, they fail to activate transcription in intact cells (11). In view of this, we wanted to determine whether the HPV 16 E2 and E2Ct proteins would form heterodimers and whether these heterodimers would be capable of inducing cell death. To ascertain whether or not heterodimers could be formed *in vitro*, we mixed a fixed amount of wild-type E2Ct, with increasing amounts of the DNA binding defective E2Ct$_{DBDm}$ protein. To facilitate the exchange of subunits, we added 3 M urea to denature both of the homodimeric proteins and then diluted the urea to 0.1 M to allow refolding and the random assortment of partners. The DNA binding activity of the refolded proteins was then determined using a gel retardation assay (Fig. 7a). As expected, refolded E2Ct bound to a labeled oligonucleotide carrying an E2 site, whereas refolded E2Ct$_{DBDm}$ showed no DNA binding activity (Fig. 7a, *lanes 3* and *4,* respectively). Adding increasing amounts of E2Ct$_{DBDm}$ to a fixed amount of E2Ct resulted in a gradual decline in DNA binding activity (Fig. 7a, *lanes 5–8*). These data show that at least in this *in vitro* assay, these E2 proteins can form heterdimers.

To investigate the formation of heterodimers in intact cells, we transiently co-transfected pWEB-E2 into HeLa cells along with increasing amounts of pWEB-E2Ct and determined the percentage of apoptotic cells exactly as described above. As expected, the pWEB-E2 plasmid induced high levels of cell death in the transfected population, whereas the pWEB-E2Ct
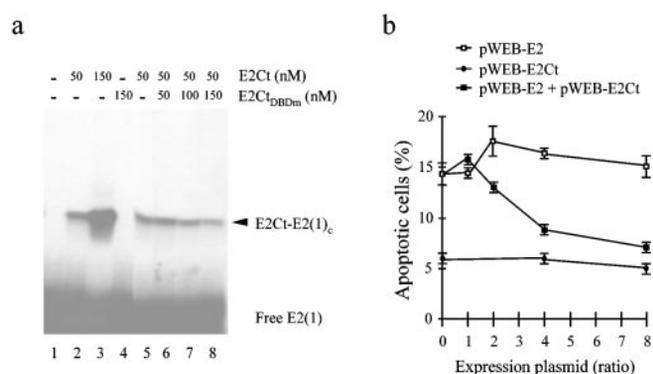
FIG. 7. **E2-E2Ct heterodimers do not induce apoptosis.** *a,* the amounts of E2Ct and E2Ct$_{DBDm}$ indicated in the figure were mixed and then denatured in 3 M urea. After refolding by dilution into 0.1 M urea, the proteins were added to labeled oligonucleotides carrying the HPV 16 E2 binding site 1 (E2(1)). Free and bound DNA was separated on a 6% polyacrylamide gel and visualized by autoradiography. The E2Ct-E2(1) complex is indicated by an *arrowhead. b,* HeLa cells were transiently transfected with pWEB-E2 and increasing amounts of the empty pWEB plasmid (*open squares*), pWEB-E2Ct and increasing amounts of pWEB (*filled circles*), or pWEB-E2 and increasing amounts of pWEB-E2Ct (*filled squares*). Apoptotic cells were identified as in Fig. 2, and the transfection was performed in duplicate and repeated three times.

plasmid had no effect (Fig. 7*b*). However, as increasing amounts of the pWEB-E2Ct plasmid were added to a transfection mixture containing pWEB-E2, the percentage of apoptotic cells in the transfected population showed a steady decline and eventually reached background levels (Fig. 7*b*). Increasing amounts of the pWEB-E2Ct$_{DBDm}$ plasmid also decreased the level of pWEB-E2-induced cell death, whereas increasing amounts of the empty pWEB plasmid had no effect (not shown). Taken together, these data suggest that heterodimers containing E2 and E2Ct form in intact cells and that these heterodimers are incapable of inducing apoptosis. Thus, it appears that the E2 dimer requires two functional N-terminal domains in order to induce cell death.

DISCUSSION

The E2 proteins from HPV 16, HPV 18, and BPV1 all have dramatic effects on the proliferation and/or survival of cervical carcinoma cell lines (12–14). We previously proposed that the HPV 16 E2 protein induces apoptosis in HPV 16-transformed SiHa cells by activating transcription of the viral E7 gene (12). In contrast, others have proposed that the HPV 18 E2 protein induces apoptosis in HPV 18-transformed HeLa cells by repressing transcription of the viral E6 and E7 genes (13). Here we have shown that the HPV 16 E2 protein induces apoptosis in two non-HPV-transformed cell lines, supporting the demonstration that the HPV 31 E2 protein appears to induce apoptosis in HPV-negative NHK cells (17). These data show that neither of the above mechanisms can be entirely correct. Either the E2 proteins induce apoptosis independently of the HPV genome or these proteins induce apoptosis via two pathways: one requiring other HPV proteins and one independent of other HPV proteins.

The E7 protein has been extensively studied, primarily as an oncoprotein, but also as an inducer of apoptosis. For example, E7 has been shown to sensitize keratinocytes to undergo both spontaneous apoptosis and apoptosis in response to tumor necrosis factor α (38). There is evidence to suggest that E7-induced apoptosis occurs via p53-dependent and p53-independent pathways (39, 40, 38). Here we have shown that in HeLa cells, overexpression of the HPV 16 E7 protein induces apoptosis. The E7-induced apoptosis can be abrogated by overexpression of the HPV 16 E6 protein or by expression of a trans-dominant negative mutant of p53. These findings strongly

suggest that the HPV 16 E7 protein induces p53-dependent apoptosis in these cells. Some controversy has surrounded the role of p53 in E2-induced cell death. For example, expression of the BPV1 E2 protein in HeLa cells has been reported to stabilize p53 (16, 13), whereas expression of the HPV 31 E2 protein in NHK cells has been reported to destabilize p53 (17). We have shown that, like E7, the HPV 16 E2 protein induces apoptosis in HeLa cells and that this apoptosis is p53-dependent. Both E7 and E2 induce apoptosis in cells that contain the HPV 16 E6 gene. Given that E6 binds to p53 and that this interaction results in a decrease in the half-life of p53 (18–21), this might seem remarkable. However, p53 activity has been demonstrated in several HPV-positive cell lines (13, 41, 42). For example, treatment of SiHa and HeLa cells with genotoxic agents results in increased nuclear p53 levels, increased binding of p53 to a p53 recognition site, and increased expression of the p53-responsive *WAF1/CIP1* gene (42).

The E2 proteins bind to specific DNA sequences and regulate viral gene expression. We have shown that the sequence-specific DNA binding activity of the HPV 16 E2 protein is not required for the induction of apoptosis in HeLa cells. In contrast, the N-terminal domain, including the transcription activation domain and the hinge region, is essential for the promotion of cell death. The E2 protein from BPV1 has been shown to arrest the growth of HeLa cells (14, 15). Growth arrest by BPV1 E2 requires a functional DNA binding domain and a functional transcription activation domain (43). These data suggest that the induction of apoptosis by the HPV 16 E2 protein and the induction of growth arrest by the BPV1 E2 protein, are brought about by two separate pathways. Presumably, growth arrest brought about by the BPV1 E2 protein requires transcriptional regulation of the integrated HPV oncogenes. Thus, BPV1 E2-induced growth arrest could be the result of transcriptional repression of the integrated E6 and E7 genes (43). However, it is important to point out that BPV1 E2 could arrest growth via the regulation of cellular genes. Interestingly, functional transcription activation domains are required for efficient repression of transcription by BPV1 E2, as well as for efficient activation of transcription (11, 43). Repression of E6 transcription would be expected to result in increased levels of p53, and this could lead to p53-dependent apoptosis (16, 13). In contrast, the induction of apoptosis by HPV 16 E2 occurs independently of its DNA binding activity and independently of the presence of integrated HPV sequences. Although at present we do not know how the HPV 16 E2 protein induces apoptosis, the mechanism probably involves changes in E2F activity. Overexpression of the HPV 16 E2 protein in SiHa cells has been shown to result in increased E2F activity (12), as has overexpression of the HPV 31 E2 protein in NHK cells (17). Like HPV 16 E2, the E2F-1 protein can induce p53-dependent apoptosis (28, 29).

Using E2 heterodimers, we have shown that two functional transcription activation domains are required for the induction of apoptosis by the HPV 16 E2 protein. Similar heterodimer experiments with BPV1 E2 and BPV1 E2-TR showed that two functional activation domains are required for efficient transcription activation (11). Why two activation domains per E2 dimer should be essential for either transcription activation or the induction of apoptosis is not known. However, the activation domains of BPV1 E2 mediate cooperative interactions between E2 dimers and bring about the formation of DNA loops (30). Two functional transcription activation domains per dimer might be required to bring about these interactions and/or contacts with other proteins involved in transcription or apoptosis.

In conclusion, although further work is required to elucidate the path that links E2 to cell death, our findings have clear

implications for the role that the loss of this protein plays in the natural history of cervical cancer. We have shown that the HPV 16 E2 protein brings about apoptosis in the absence of other HPV gene products and that this E2-induced apoptosis is p53-dependent. Integration of the HPV genome into the host chromosome and the consequent disruption of the E2 gene removes this proapoptotic signal. Because the integrated HPV sequences continue to produce the E6 and E7 proteins, these cells continue to proliferate and are likely to form cervical tumors.

REFERENCES

1. zur Hausen, H. (1991) *Virology* **184,** 9–13
2. Campo, M. S., Jarrett, W. F. H., Barron, R., O'Neil, B. W., and Smith, K. T. (1992) *Cancer Res.* **52,** 6898–6904
3. Campo, M. S., O'Neill, B. W., Barron, R. J., and Jarrett, W. F. H. (1994) *Carcinogenesis* **15,** 1597–1601
4. Crook, T., and Vousden, K. H. (1996) in *Papillomavirus Reviews: Current Research on Papillomaviruses* (Lacey, C., ed) pp. 55–60, Leeds University Press, Leeds, United Kingdom
5. Baker, C. C., Phelps, W. C., Lindgren, V., Braun, M. J., Gonda, M. A., and Howley, P. M. (1987) *J. Virol.* **61,** 962–971
6. Romanczuk, H., and Howley, P. M. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 3159–3163
7. Thierry, F. (1996) in *Papillomavirus Reviews: Current Research on Papillomaviruses* (Lacey, C., ed) pp. 21–29, Leeds University Press, Leeds, United Kingdom
8. Bouvard, V., Storey, A., Pim, D., and Banks, L. (1994) *EMBO J.* **13,** 5451–5459
9. Kovelman, R., Bilter, G. K., Glezer, E., Tsou, A. Y., and Barbosa, M. S. (1996) *J. Virol.* **70,** 7549–7560
10. Giri, I., and Yaniv, M. (1988) *EMBO J.* **7,** 2823–2829
11. Barsoum, J., Prakash, S. S., Han, P., and Androphy, E. J. (1992) *J. Virol.* **66,** 3941–3945
12. Sanchez-Perez, A.-M., Soriano, S., Clarke, A. R., and Gaston, K. (1997) *J. Gen. Virol.* **78,** 3009–3018
13. Desaintes, C., Demeret, C., Goyat, S., Yaniv, M., and Thierry, F. (1997) *EMBO J.* **16,** 504–514
14. Hwang, E.-S., Riese, D. J., II, Settleman, J., Nilson, L. A., Honig, J., Flynn, S., and DiMaio, D. (1993) *J. Virol.* **67,** 3720–3729
15. Dowhanick, J. J., McBride, A. A., and Howley, P. M. (1995) *J. Virol.* **69,** 7791–7799
16. Hwang, E.-S., Naeger, L. K., and DiMaio, D. (1996) *Oncogene* **12,** 795–803
17. Frattini, M. G., Hurst, S. D., Lim, H. B., Swaminathan, S., and Laimins, L. A. (1997) *EMBO J.* **16,** 318–331
18. Werness, B. A., Levine, A. J., and Howley, P. M. (1990) *Science* **248,** 76–79
19. Scheffner, M., Werness, B. A., Huibregtse, J. M., Levine, A. J., and Howley, P. M. (1990) *Cell* **63,** 1129–1136
20. Lechner, M. S., Mack, D. H., Finicle, A. B., Crook, T., Vousden, K. H., and Laimins, L. A. (1992) *EMBO J.* **11,** 3045–3052
21. Hubbert, N. L., Sedman, S. A., and Schiller, J. T. (1992) *J. Virol.* **66,** 6237–6241
22. Gottlieb, T. M., and Oren, M. (1998) *Semin. Cancer Biol.* **8,** 359–368
23. Dyson, N., Howley, P. M., Münger, K., and Harlow, E. (1989) *Science* **243,** 934–937
24. Hu, T., Ferril, S. C., Snider, A.-M., and Barbosa, M. S. (1995) *Int. J. Oncol.* **6,** 167–174
25. Boyer, S. N., Wazer, D. E., and Band, V. (1996) *Cancer Res.* **56,** 4620–4624
26. Jones, D. L., and Münger, K. (1997) *J. Virol.* **71,** 2905–2912
27. Jones, D. L., Thompson, D. A., and Münger, K. (1997) *Virology* **239,** 97–107
28. Wu, X., and Levine, A. J. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 3602–3606
29. Qin, X.-Q., Livingston, D. M., Kaelin, W. G., Jr., and Adams, P. D. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 10918–10922
30. Knight, J. D., Li, R., and Botchan, M. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 3204–3208
31. Bartholomew, J. S., Glenville, S., Sarkar, S., Burt, D. J., Stanley, M. A., Ruiz-Cabello, F., Chengang, J., Garrido, F., and Stern, P. L. (1997) *Cancer Res.* **57,** 937–942
32. Chirillo, P., Pagano, S., Natoli, G., Puri, P. L., Burgio, V. L., Balsano. C., and Levrero, M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 8162–8167
33. Crook, T., Wrede, D., and Vousden, K. H. (1991) *Oncogene* **6,** 873–875
34. Farmer, G., Bargonetti, J., Zhu, H., Friedman, P., Prywes, R., and Prives, C. (1992) *Nature* **358,** 83–86
35. Huibregtse, J. M., Scheffner, M., and Howley, P. M. (1991) *EMBO J.* **10,** 4129–4135
36. Hegde, R. S., and Androphy, E. J. (1998) *J. Mol. Biol.* **284,** 1479–1489
37. Hegde, R. S., Grossman, S. R., Laimins, L. A., and Sigler, P. B. (1992) *Nature* **359,** 505–512
38. Stöppler, H. Stöppler, M. C. Johnson, E., Simbulan-Rosenthal, C. M., Smulson, M. E. Iyer, S., Rosenthal, D. S., and Schlegel, R. (1998) *Oncogene* **17,** 1207–1214
39. Howes, K. A., Ransom, N., Papermaster, D. S., Lasudry, J. G. H., Albert, D. M., and Windle, J. J. (1994) *Genes Dev.* **8,** 1300–1310
40. Pan, H., and Griep, A. E. (1994) *Genes Dev.* **8,** 1285–1299
41. Scheffner, M., Münger, K., Byrne, J. C., and Howley, P. M. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 5523–5527
42. Butz, K., Shahabeddin, L., Geisen, C., Spitkovsky, D., Ullmann, A., and Hoppe-Seyler, F. (1995) *Oncogene* **10,** 927–936
43. Goodwin, E. C., Naeger, L. K., Breiding, D. E., Androphy, E. J., and DiMaio, D. (1998) *J. Virol.* **72,** 3925–3934

The Human Papillomavirus (HPV) 16 E2 Protein Induces Apoptosis in the Absence of Other HPV Proteins and via a p53-dependent Pathway

Kenneth Webster, Joanna Parish, Maya Pandya, Peter L. Stern, Anthony R. Clarke and Kevin Gaston

Access the most updated version of this article at http://www.jbc.org/content/275/1/87

Alerts:

- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 41 references, 23 of which can be accessed free at
http://www.jbc.org/content/275/1/87.full.html#ref-list-1