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Human papillomavirus 16 E6 expression disrupts the p53-mediated cellular response to DNA damage

(cell cycle/carcinogenesis/cervical carcinoma/DNA replication)

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ABSTRACT **Infection with certain types of human papillomaviruses (HPV) is highly associated with carcinomas of the human uterine cervix. However, HPV infection alone does not appear to be sufficient for the process of malignant transformation, suggesting the requirement of additional cellular events. After DNA damage, normal mammalian cells exhibit G_1 cell-cycle arrest and inhibition of replicative DNA synthesis. This mechanism, which requires wild-type p53, presumably allows cells to undertake DNA repair and avoid the fixation of mutations. We directly tested whether the normal response of cervical epithelial cells to DNA damage may be undermined by interactions between the E6 protein expressed by oncogenic HPV types and wild-type p53. We treated primary keratinocytes with the DNA-damaging agent actinomycin D and demonstrated inhibition of replicative DNA synthesis and a significant increase in p53 protein levels. In contrast, inhibition of DNA synthesis and increases in p53 protein did not occur after actinomycin D treatment of keratinocytes immortalized with HPV16 E6/E7 or in cervical carcinoma cell lines containing HPV16, HPV18, or mutant p53 alone. To test the effects of E6 alone on the cellular response to DNA damage, HPV16 E6 was expressed in the carcinoma cell line RKO, resulting in undetectable baseline levels of p53 protein and loss of the G_1 arrest that normally occurs in these cells after DNA damage. These findings demonstrate that oncogenic E6 can disrupt an important cellular response to DNA damage mediated by p53 and may contribute to the subsequent accumulation of genetic changes associated with cervical tumorigenesis.**

Clinical and epidemiologic studies have implicated human papillomavirus (HPV) infection in the development of cervical carcinoma (1). Only a subgroup of the 67 distinct HPV types identified to date appear capable of infecting the lower genital tract. The high-risk HPVs (most notably HPV16 and HPV18) are associated with high-grade squamous intraepithelial lesions and invasive cervical carcinomas, whereas the low-risk types are found more often in low-grade lesions (1). In the majority of primary cervical carcinomas and cervical tumor cell lines studied, the viral genomes of high-risk HPV types are integrated into the host genome, often disrupting the *E1* and *E2* genes and allowing active expression of the *E6* and *E7* genes (2–4). DNA encoding E6 and E7 has been found to immortalize primary cells *in vitro*, but a fully transformed phenotype rarely appears until after numerous passages (5, 6), suggesting that additional events are required. In addition, although infection with high-risk HPV types may be quite common (7), only a small percentage of infected women develop invasive cervical cancer. These observations are consistent with the multistep theory of tumorigenesis, in which the accumulation of genetic alterations plays a central role (8). Thus, although viral infection alone may not be sufficient for the process of malignant transformation, expression of the E6 and E7 proteins may facilitate cervical tumor development by predisposing infected cells to the accumulation of genetic lesions.

Somatic acquisition of genetic lesions may occur after replication of damaged DNA, allowing these alterations to become fixed in daughter cells. Normal mammalian cells exhibit G_1 and G_2 cell-cycle arrest in response to DNA damage, presumably allowing cells time to repair the damage before initiating replicative DNA synthesis or mitosis (9, 10). The arrest of cells in G_1 after exposure to DNA strand-breaking agents, such as ionizing radiation (IR) or actinomycin D (ActD), is accompanied by a transient increase in the level of wild-type p53 (wtp53) protein (11). In contrast, cells lacking wtp53 or expressing mutant p53 fail to exhibit G_1 arrest and the associated inhibition of DNA synthesis after DNA damage (11, 12). Transfection of wtp53 into cells lacking endogenous p53 genes results in the restoration of G_1 arrest after DNA damage, whereas introduction of mutant p53 into cells containing wtp53 genes results in the loss of G_1 arrest after DNA damage (12). Furthermore, embryonic mouse fibroblast cells, either homozygous or hemizygous for wtp53, undergo G_1 arrest after DNA damage, whereas cells lacking two functional copies of wtp53 do not (13).

Previous studies have shown that E6 proteins translated from high-risk HPV *E6* genes are able to bind wtp53 and mediate p53 degradation *in vitro* through a ubiquitin-mediated mechanism (14, 15). The normal cellular response to DNA damage may be disrupted by high-risk HPV infection, perhaps mediated solely by E6 through an interaction with wtp53. This interaction between oncogenic E6 and wtp53 suggests a plausible mechanism for the enhanced accumulation of genetic alterations necessary for cervical tumor progression that occur in the setting of high-risk HPV infection. To assess the consequences of high-risk HPV E6 expression on cell-cycle changes after DNA damage, we compared the response of normal keratinocytes to DNA damage with the response of HPV E6/E7 immortalized keratinocytes and several cervical carcinoma-derived cell lines expressing either oncogenic E6 or mutant p53. In addition, we introduced E6 into the colorectal carcinoma cell line RKO, which normally expresses wtp53 and exhibits a G_1 arrest after DNA damage. All cell lines were examined for modulation of p53 protein levels and mRNA expression after DNA damage.

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Abbreviations: HPV, human papillomavirus; wtp53, wild-type p53; ActD, actinomycin D; IR, ionizing radiation.

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We found that oncogenic E6 expression, even in the absence of other HPV proteins, consistently abolishes the G_1 arrest associated with the normal cellular response to DNA damage. Moreover, E6 expression is associated with a dramatic decrease in p53 protein levels. Our results support the proposal that wtp53 is a cell-cycle check-point determinant involved in the DNA-damage response (12). The ability of the E6 protein to eliminate this important cellular response may, therefore, contribute to the subsequent accumulation of genetic changes responsible for cervical tumorigenesis.

MATERIALS AND METHODS

Cells, DNA Damage, and Cell-Cycle Analysis. Normal ectocervical epithelium, obtained through The Johns Hopkins Hospital, Department of Pathology, was used to establish primary cervical keratinocyte cultures, as described (16). Cervical tissue fragments were obtained from uteri removed from patients with multiple uterine leiomyomata. None of these patients had any history of cervical disease. Normal primary foreskin keratinocyte (Clonetics, San Diego) and primary cervical keratinocyte cultures were maintained in KGM medium (Clonetics). Foreskin keratinocyte lines were immortalized with various combinations of HPV open reading frames [10b (HPV16 E6/HPV11 E7) and 8217 (HPV16 E6/E7)]. The HPV sequences were amplified and cloned into the rat β-actin expression vector (pJ6Ω). Human foreskin keratinocytes at the fourth passage were transfected with 5 μg of DNA using Lipofectin (GIBCO), and differentiation-resistant foci were selected (A.T.L., unpublished data). The clones were cultured in a mixture of KGM medium/Dulbecco's modified Eagle's medium (DMEM), 3:1, containing 10% (vol/vol) fetal bovine serum (GIBCO). Cervical carcinoma cell lines (C33A, C4II, CaSki, HeLa, HT-3, ME180, and SiHa) were obtained from the American Type Culture Collection and cultured in DMEM/10% (vol/vol) fetal bovine serum. RKO cells were provided by Bert Vogelstein (Johns Hopkins University) and maintained in McCoy's 5A medium/10% (vol/vol) fetal calf serum. To evaluate perturbations of cell-cycle progression after DNA damage, cells were cultivated in 8-well chamber slides (Nunc) to logarithmic-phase growth and then treated with various concentrations of ActD (0 nM–4.5 nM) for 24 hr at 37°C. Cells were then washed in two changes of Hanks' balanced salt solution (GIBCO), cultured for an additional 4 hr in the presence of 2.5 μCi of [^{3}H]thymidine per ml (1 Ci = 37 GBq), fixed in methanol at 4°C for 1 min, dipped in NTB-2 photographic emulsion (Kodak), and exposed for 24–48 hr. S-phase indices (percentage of cells in S phase) were derived by counting 200 cells per well in at least five high-power fields and determining the fraction of labeled cells. Alternatively, cells were irradiated in a ^{137}Cs γ-irradiator at 1 Gy/min for 4 min. Cell-cycle status was determined by dual-parameter flow cytometric analysis of cells labeled with 10 μM 5-bromodeoxyuridine for 4 hr at 17 hr after irradiation and subsequently stained, for DNA synthesis, with a fluorescein isothiocyanate-conjugated anti-5-bromodeoxyuridine antibody and for DNA content with propidium iodide, as described (11).

RKO Transfections. The PCR was used to amplify the HPV16 E6 open reading frame (HPV16 map positions 42–527 bp) from Cx10, a primary cervical carcinoma obtained from the Johns Hopkins Surgical Pathology Tumor Bank. The pCMV-E6 expression construct was obtained by cloning the amplified products into a unique *Bam*HI restriction site located downstream of the cytomegalovirus promoter in plasmid pCMVneo (provided by Bert Vogelstein). The *E6* insert was sequenced in its entirety to exclude *Taq* polymerase error. Logarithmically growing RKO cells were transfected with pCMV-E6 or pCMVneo alone using Lipofectin, essentially as described by the manufacturer. Polyclonal populations of *E6* transfectants or control transfectants were obtained by G418 selection (1.0 mg/ml) and then cloned by limiting dilution in the presence of G418 at 0.5 mg/ml.

Immunoblot Analysis. Subconfluent monolayer cultures were either treated with ActD at a concentration of 0.5 nM for 24 hr, 4 Gy of IR, or left untreated. For immunoblot analysis of p53 protein levels, cells were lysed in sample buffer (2% SDS/5% glycerol/5% 2-mercaptoethanol in 0.0625 M Tris, pH 6.8), 24 hr after drug treatment or 4 hr after IR treatment, immediately boiled for 10 min, and stored at −80°C. Samples (100 μg, as determined by an amido black quantification assay) were analyzed by SDS/PAGE and transferred to nitrocellulose (Hybond-ECL, Amersham) with a semi-dry electroblotter (Millipore). A mixture of mouse monoclonal antibodies mAb-1801 (17) and mAb-421 (18) (Oncogene Science, Mineola, NY) was used in conjunction with a goat anti-mouse horseradish peroxidase-conjugated secondary antibody (Pierce) for detection of p53. Enhanced chemiluminescence (ECL, Amersham) was used for final detection of the immunoreactive protein, as described by the manufacturer.

RESULTS

Effect of DNA Damage on Replicative DNA Synthesis in Normal, HPV16 E6/E7 Immortalized, and HPV-Positive Cervical Carcinoma Cells. The normal cellular response after exposure to the DNA intercalating agent ActD, which at sublethal doses causes DNA strand breaks (19), is inhibition of DNA synthesis via G_1 arrest (9–11). Replicative DNA synthesis was inhibited in primary foreskin (K-11) and cervical keratinocyte (PCx-7) cultures treated with ActD at the nonlethal dose of 0.5 nM (Fig. 1 *A* and *B* and Fig. 2*A*). Inhibition of DNA synthesis was not observed after treatment with the same level of ActD in the HPV16 E6/E7 immortalized foreskin keratinocyte line 8217 (Fig. 2*A*), the HPV 18-positive cervical carcinoma-derived line HeLa (Fig. 1 *C* and *D* and Fig. 2*A*), or the HPV-negative cervical carcinoma line C33A, which harbors a mutant copy of p53 (Fig. 2*A*). Additional keratinocyte cultures and cervical carcinoma cell lines of various HPV and p53 status were evaluated for inhibition of DNA synthesis after ActD treatment (Table 1). Consistently, cells containing p53 mutations or high-risk HPV DNA failed to optimally inhibit DNA synthesis after DNA damage.

p53 Protein and mRNA Levels in Cervical Cells After DNA Damage. G_1 arrest and the subsequent block of replicative DNA synthesis in response to DNA damage are temporally associated with a transient increase in wtp53 protein levels in several mammalian cell lines (11–13). The E6 protein expressed by oncogenic HPVs binds to wtp53 protein (14, 15) and mediates p53 degradation *in vitro* via a ubiquitin-dependent mechanism (20). Immunoblot analysis was used to determine whether the presence of HPV E6 affected the increased levels of wtp53 protein associated with the normal cellular response to DNA damage. ActD treatment of primary keratinocyte cultures K-11 and PCx-7 resulted in increased wtp53 protein, whereas no increases in p53 protein levels were seen in the HPV16 E6/E7 immortalized keratinocyte lines 10b and 8217 or in the HPV-positive cervical carcinoma cell lines HeLa and SiHa, which also express E6 (Fig. 2*B*). Three additional cervical carcinoma cell lines, which express the E6 oncoprotein (C4II, CaSki, and Me180) behaved in a similar manner after DNA damage (Table 1). Immunoblot analysis of ActD-treated C33A and HT-3 cells confirmed earlier reports that p53 protein levels do not increase in response to DNA damage in cell lines containing mutant p53 genes (Fig. 2*B*) (11, 12). ActD treatment did not affect p53 mRNA levels in any cell lines tested (data not shown), confirming previous reports that increases in wtp53 protein after IR or ActD-mediated DNA damage may result from a posttranscriptional mechanism (11).

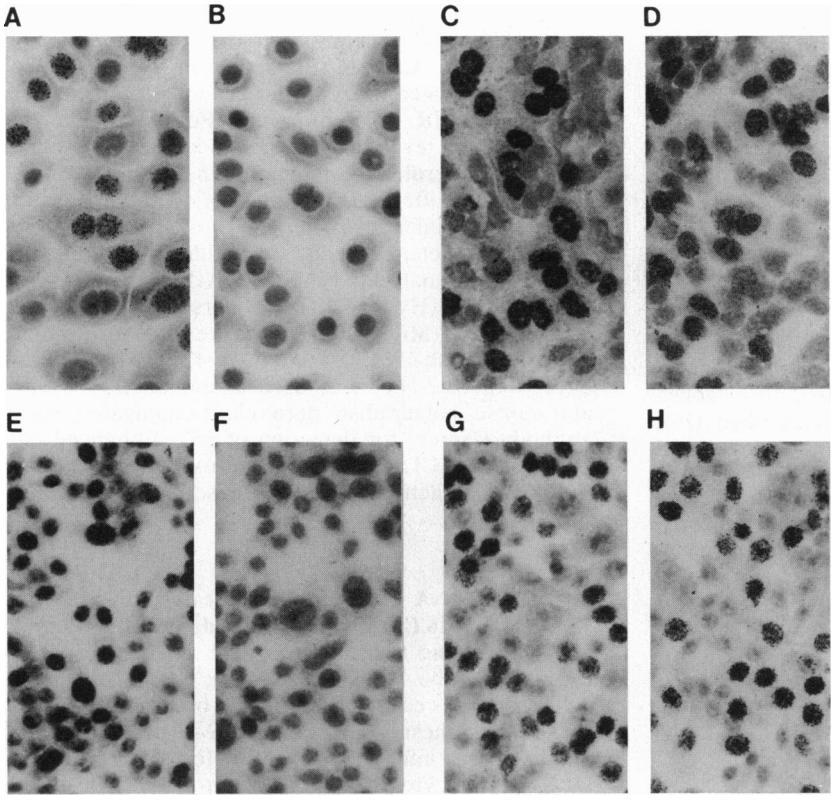

FIG. 1. Photomicrograph of the DNA-damage response assay using the following cells: the human primary cervical keratinocyte culture PCx-7 (*A* and *B*), the HPV18-positive cervical carcinoma line HeLa (*C* and *D*), RCneo.1, RKO cells transfected with pCMVneo (*E* and *F*), and RC10.1, RKO cells transfected with pCMV-E6 (*G* and *H*). Cells were treated with either 0.5 nM ActD (*B*, *D*, *F*, and *H*) or left untreated (*A*, *C*, *E*, and *G*). Silver grains appearing over the nuclei of cells indicate active DNA synthesis. (×160.)

Cell-Cycle Progression of RKO Cells Transfected with HPV16 E6 After DNA Damage. Because all E6-expressing cell lines described above express other viral gene products in addition to E6, we sought to determine whether E6 expression alone might disrupt the cellular response to DNA damage. RKO cells were used in transfection studies assessing the effect of HPV16 E6 alone on the DNA-damage response. These cells contain wtp53 and display G_1 arrest and induction of wtp53 protein after DNA damage (12). Inhibition of DNA synthesis was observed in parental RKO cells and in RKO cells transfected with control vector pCMVneo (RCneo) after treatment with 0.5 nM ActD (Fig. 1 *E* and *F*). Four separate HPV16 *E6*-transfected RKO clonal cell lines (designated RC10.1–RC10.4) were shown to express E6 mRNA by RNA blot analysis (data not shown). All four clones treated with 0.5 nM ActD failed to inhibit DNA synthesis (Fig. 1 *G* and *H* and Fig. 3*A*). In addition, a polyclonal population of HPV16 *E6*-transfected RKO cells representing pooled stable neomycin-resistant clones showed an intermediate degree of DNA-synthesis inhibition in response to ActD (data not shown).

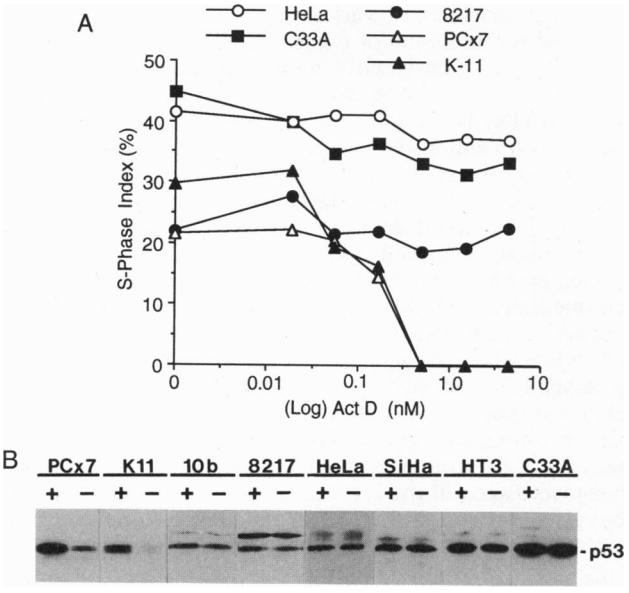

FIG. 2. Alteration of replicative DNA synthesis and p53 protein levels in selected cell lines after ActD exposure. (*A*) Primary human cervical and foreskin keratinocyte cultures PCx-7 and K-11; HPV16 E6/E7 immortalized line 8217; and cervical carcinoma-derived lines HeLa (HPV18) and C33A (mutant p53) after treatment with various ActD concentrations (0 nM–4.5 nM) for 24 hr and labeled with [^{3}H]thymidine. (*B*) Immunoblot analysis of p53 protein levels in untreated cells (−) described above or after treatment for 24 hr with 0.5 nM ActD (+). Cervical carcinoma lines SiHa (HPV16) and HT-3 (mutant p53) are also shown.

Disruption of the normal cellular response to DNA damage by E6 was not limited to treatment with ActD. IR was used as an alternative means of inducing DNA damage. Cells were analyzed by flow cytometry, as described above, and changes in the G_1/S ratio were assessed. The G_1/S ratio increases with arrest in G_1 after IR. The G_1/S ratio increased significantly after treatment of RCneo.1 cells, but this increase was not seen in any of the clonal cell lines transfected with HPV16 *E6* or in the colorectal carcinoma cell line SW480 expressing mutant p53 (Fig. 3*B*).

p53 Protein and mRNA Levels in *E6*-Transfected RKO Cells After DNA Damage. Exposure of RKO and RCneo.1 cells to 4 Gy of IR or 0.5 nM ActD increased the levels of wtp53 protein. p53 protein was undetectable in the four untreated RC10 clones and remained undetectable after ActD exposure or IR (Fig. 3*C*). Treatment with ActD or IR had no effect on p53 or E6 mRNA levels (data not shown).

DISCUSSION

Transient inhibition of replicative DNA synthesis in mammalian cells suffering DNA damage may provide substantial protection against environmental insults threatening genome integrity. Loss of this physiologic response might potentially

Table 1. Status of p53 gene, HPV infection, and response to DNA damage in selected cell lines

Cell lines	HPV DNA and RNA	p53*	ActD treatment,† % S-phase		p53 protein‡
			Untreated	0.5 nM	
Normal					
PCx-7		WT	21.4 (0.7)	<1.0	I
K-1		WT	9.3 (0.9)	<1.0	I
K-11		WT	29.5 (0.4)	<1.0	I
K-14		WT	25.6 (1.9)	<1.0	I
Immortalized					
10b	HPV16 E6/ HPV11 E7	WT	20.2 (1.2)	23.6 (1.0)	NI
8217	HPV16 E6/E7	WT	21.9 (1.9)	18.5 (0.6)	NI
Tumor derived					
C33A		Mutant	44.8 (0.6)	33.2 (1.5)	NI
HT-3		Mutant	40.2 (2.9)	24.7 (0.8)	
C4II	HPV18	WT	30.9	32.0	NI
CaSki	HPV16	WT	50.2	40.4	NI
HeLa	HPV18	WT	41.8 (1.3)	36.6 (1.0)	NI
ME180	HPV39§	WT	19.3	22.3	NI
SiHa	HPV16	WT	24.6	19.0	NI

*WT, wild type; Mutant, C33A (Arg-273 → Cys) and HT-3 (Gly-245 → Val).
†DNA-damage response assay. S-phase index (% S-phase) was determined for cells treated for 24 hr with 0.5 nM ActD, as well as for untreated cells. S-phase indices displayed for C4II, CaSki, ME180, and SiHa cells represent mean values from two independent drug exposures. The remaining S-phase indices represent mean values derived from a minimum of three independent drug exposures (SE in parentheses).
‡Immunoblot analysis of cells treated with 0.5 nM ActD. I, induction of p53 protein; NI, no induction.
§HPV39-related type.

render a cell highly susceptible to the accumulation of multiple genetic lesions that underlie neoplastic transformation and tumor progression. Recent data have suggested a central role for wtp53 protein in inhibiting replicative DNA synthesis after DNA damage (11–13) and on maintaining genome integrity (21, 22). Inhibition of replicative DNA synthesis in normal cervical epithelial cells after ActD exposure was associated with an elevation in wtp53 protein levels. In contrast, DNA synthesis was not inhibited by ActD-mediated DNA damage in keratinocytes immortalized with HPV16 E6/E7 or in cervical carcinoma cell lines expressing E6 or mutant p53. Thus, our studies of the response of normal and neoplastic human uterine cervical epithelial cells to the DNA-damaging agent ActD are consistent with the critical role of wtp53 protein in the physiologic response to DNA damage. Expression of E6 alone in RKO cells not only disrupted the normal inhibition of DNA synthesis exhibited by these cells after DNA damage but also significantly reduced the baseline levels of wtp53 protein. These findings provide evidence that E6 itself, most likely through an interaction with wtp53, is responsible for abolishing the normal cellular response to DNA damage in the setting of high-risk HPV infection.

The similarity of response to DNA damage between cell lines expressing mutant p53 or oncogenic E6 suggests that in this setting, E6/wtp53 interactions may be functionally equivalent to p53 mutations. That cells with mutant p53 genes and cells infected with HPV18 (HeLa) both fail to induce the p53-dependent *GADD45* gene in response to irradiation (13) supports this concept. The presence of p53 mutations in HPV-negative cervical carcinoma cell lines, such as C33A and HT-3, further supports the notion that mutations of p53 may functionally replace the interaction between wtp53 and E6 (23, 24). Recent data, however, suggest that p53 mutations may also occasionally occur in HPV-positive cervical carcinomas (ref. 25 and T.D.K., unpublished data). These mutations may confer an additional growth advantage to affected cells (26). Furthermore, p53 gene mutations are not always found in HPV-negative cervical carcinomas (25). Possibly, transient inactivation of wtp53 function during high-risk HPV infection might promote genetic alterations, generating a stably transformed cell that no longer requires the presence of the HPV genome for maintenance of its neoplastic phenotype ("hit and run oncogenesis") (27). Alternatively, HPV-negative, wtp53-expressing cervical tumors may arise through pathways entirely independent of the genes involved in this or other DNA-damage response pathways. Also, inactivation of other genes in the p53-mediated DNA-damage response pathway may occur. For example, the radiation-inducible gene *GADD45* has been demonstrated to be a target of transcriptional activation by wtp53 and, thus, may serve as such a target for inactivation during tumor development (13).

Clinical and epidemiological studies have implicated involvement of HPV infection in cervical tumorigenesis for many years (1). We are now beginning to understand the molecular mechanisms and consequences of HPV infection. Interactions between viral oncoproteins and cellular tumor-suppressor genes undoubtedly play a critical role in this process. Previously, *in vitro* interactions of p53 with several oncoproteins from DNA tumor viruses, including adenovirus E1b and simian virus 40 large T antigen, have been documented. Previous studies of E6/wtp53 interactions *in vitro* have provided several important clues to the functional consequences of this protein–protein association (14, 15, 20). In addition, recent *in vivo* transfection experiments show that coexpression of simian virus 40 large T antigen or HPV16/18 E6 and wtp53 interferes with the transcriptional regulatory properties of p53 (28, 29). The DNA-damage response assay reported here provides a model system in which to study the phenotypic consequences of E6 expression in living cells.

Our findings suggest that oncogenic E6/wtp53 interactions may disrupt an important cellular response to DNA damage. Inhibition of replicative DNA synthesis in response to DNA damage may be a protective physiological response to environmental insults that affect chromatin structure and the DNA template in normal cells. Loss of this response may render cells highly susceptible to the accumulation of multiple genetic lesions associated with carcinogenesis and tumor progression. HPV-infected cells expressing E6 may be predisposed to acquisition of genetic alterations in a manner similar to that observed in cells with mutant p53. Recently, Livingstone *et al.* (21) have shown that cells with mutant p53

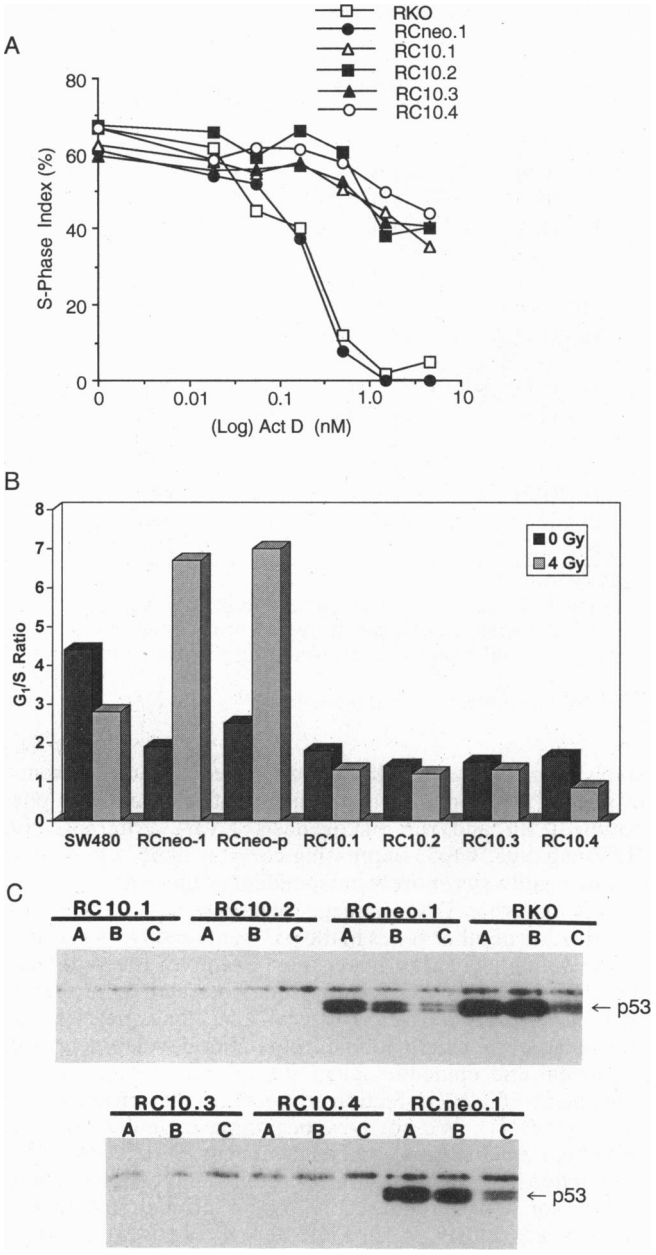

FIG. 3. (*A*) Alteration of replicative DNA synthesis in E6-expressing RC10 clones (RC10.1–RC10.4) and controls (parental RKO cells and RCneo.1, RKO cells transfected with pCMVneo) after exposure to various concentrations of ActD (0 nM–4.5 nM) for 24 hr and labeled with [^{3}H]thymidine. (*B*) Cell-cycle changes in E6-expressing RC10 clones, RCneo-p (polyclonal pCMVneo-transfected RKO line), RCneo.1, and SW480 (containing mutant p53) at 24 hr after exposure to 4 Gy of IR. Changes in cell-cycle status were assessed by flow cytometric analysis and are expressed as G_1/S ratios. (*C*) Immunoblot analysis of p53 protein levels in the E6-expressing RC10 clones, RCneo.1, and untransfected RKO cells after treatment with either 0.5 nM ActD (lanes A), 4 Gy of IR (lanes B), or untreated (lanes C).

display altered cell-cycle arrest and an increased frequency of gene amplification. Furthermore, Yin *et al.* (22) have shown that introduction of wtp53 into cells lacking p53 restores cell-cycle control and reduces the frequency of gene amplification. Amplification of specific genes is a well-recognized phenomenon associated with some human cancers. That certain viral infections may predispose cells to genetic alterations is consistent with the notion of multistep tumorigenesis (8) and may be particularly crucial for uterine cervix tumor progression, where HPV infection appears to serve as the initiating event in most, if not all, cases.

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1. zur Hausen, H. (1989) in *Advances in Viral Oncology*, ed. Klein, G. (Raven, New York), Vol. 8, pp. 1–26.
2. Schwarz, E., Freese, U. K., Gissmann, L., Mayer, W., Roggenbuck, B., Stremlau, A. & zur Hausen, H. (1985) *Nature (London)* **314**, 111–114.
3. Smotkin, D. & Wettstein, F. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4680–4684.
4. Baker, C., Phelps, W., Lindgren, V., Braun, M., Gonda, M. & Howley, P. (1987) *J. Virol.* **61**, 962–971.
5. Hawley-Nelson, P., Vousden, K. H., Hubbert, N. L., Lowy, D. R. & Schiller, J. T. (1989) *EMBO J.* **8**, 3905–3910.
6. Münger, K., Phelps, W., Bubb, V., Howley, P. & Schlegel, R. (1989) *J. Virol.* **63**, 4417–4421.
7. Young, L., Bevan, I., Johnson, M., Blomfield, P., Bromidge, N., Maitland, N. & Woodman, C. (1989) *Br. Med. J.* **298**, 14–18.
8. Fearon, E. R. & Vogelstein, B. (1990) *Cell* **61**, 759–767.
9. Painter, R. B. & Young, B. R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7315–7317.
10. Denekamp, J. (1986) *Int. J. Radiat. Biol.* **49**, 357–380.
11. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B. & Craig, R. W. (1991) *Cancer Res.* **51**, 6304–6311.
12. Kuerbitz, S., Plunkett, B., Walsh, W. & Kastan, M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 7491–7495.
13. Kastan, M., Zhan, Q., El-Deiry, W., Carrier, F., Jacks, T., Walsh, W., Plunkett, B., Vogelstein, B. & Fornace, A. (1992) *Cell* **71**, 587–598.
14. Werness, B. A., Levine, A. J. & Howley, P. M. (1990) *Science* **248**, 76–79.
15. Crook, T., Tidy, J. A. & Vousden, K. H. (1991) *Cell* **67**, 547–556.
16. Woodworth, C. D., Waggoner, S., Barnes, W., Stoler, M. H. & DiPaolo, J. A. (1990) *Cancer Res.* **50**, 3709–3715.
17. Banks, L., Matlashewski, G. & Crawford, L. (1986) *Eur. J. Biochem.* **159**, 529–534.
18. Harlow, E., Crawford, L. V., Pim, D. C. & Williamson, N. M. (1981) *J. Virol.* **39**, 861–869.
19. Wassermann, K., Markovits, J., Jaxel, C., Capranico, G., Kohn, K. W. & Pommier, Y. (1990) *Mol. Pharmacol.* **38**, 38–45.
20. Scheffner, M., Werness, B. A., Huibregtse, J. M., Levine, A. J. & Howley, P. M. (1990) *Cell* **63**, 1129–1136.
21. Livingstone, L., White, A., Sprouse, J., Livanos, E., Jacks, T. & Tlsty, T. (1992) *Cell* **70**, 923–934.
22. Yin, Y., Tainsky, M., Bischoff, F., Strong, L. & Wahl, G. (1992) *Cell* **70**, 937–948.
23. Scheffner, M., Münger, K., Byrne, J. C. & Howley, P. M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5523–5527.
24. Crook, T., Wrede, D. & Vousden, K. H. (1991) *Oncogene* **6**, 873–875.
25. Fujita, M., Inoue, M., Tanizawa, O., Iwamoto, S. & Enomoto, T. (1992) *Cancer Res.* **52**, 5323–5328.
26. Crook, T. & Vousden, K. H. (1992) *EMBO J.* **11**, 3935–3940.
27. Iwasaka, T., Hayashi, Y., Yokoyama, M., Hara, K., Matsuo, N. & Sugimori, H. (1992) *Acta Obstet. Gynecol. Scand.* **71**, 219–223.
28. Mietz, J., Unger, T., Huibregtse, J. & Howley, P. (1992) *EMBO J.* **11**, 5013–5020.
29. Lechner, M., Mack, D., Finicle, A., Crook, T., Vousden, K. & Laimins, L. (1992) *EMBO J.* **11**, 3045–3051.