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Repression of Endogenous p53 Transactivation Function in HeLa Cervical Carcinoma Cells by Human Papillomavirus Type 16 E6, Human mdm-2, and Mutant p53

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Somatic mutations in the p53 tumor suppressor gene represent the single most common genetic alteration observed in human cancers. Interestingly, the great majority of malignant tumors of the cervix uteri contain wild-type p53 alleles together with the DNA of specific types of human papillomaviruses (HPVs), while the small portion of HPV-negative cervical carcinomas often carry alterations in the p53 tumor suppressor gene. Transcriptional activation of yet-undefined cellular regulatory genes has been implicated to play a key role for the tumor-suppressive activity of wild-type p53, as mutant p53 in general has lost the activity to stimulate p53-responsive reporter plasmids. The detection of DNA-binding-competent and transcriptionally active p53 protein in HeLa cervical carcinoma cells enabled us to investigate the in vivo effects of putative modulators on endogenous p53 function in cervical cancer cells. We show that the transcriptional stimulatory activity of HeLa cell p53 is strongly repressed by overexpression of E6 protein from oncogenic HPV type 16 (HPV16) but is not influenced by low-risk HPV6 E6. Similar to HPV16 E6, cellular oncoproteins such as mutant p53 or the product of the human *mdm-2* gene also negatively interfere with p53-mediated transactivation in HeLa cells. Our findings indicate that, within a cervical cancer cell, the expression of E6 protein from high-risk HPV16, but not from low-risk HPV6, can lead to the same functional consequences as a mutation of the p53 gene. These results could provide a biochemical basis for the inverse correlation between the presence of HPV sequences and somatic mutations of the p53 gene in cervical carcinomas.

Deletions or mutations in the p53 tumor suppressor gene (9, 12) are observed in a large number of different forms of human malignancies. For example, approximately 75% of colon carcinomas, 40% of breast cancers, and 50% of lung cancers contain p53 mutations (15, 24). In contrast, only a small portion of human cervical carcinoma biopsies and cell lines derived thereof exhibit alterations in the p53 gene. Interestingly, cervical cancer cells containing integrated human papillomavirus (HPV) sequences have been reported to possess wild-type p53 alleles, while HPV-negative cell lines usually exhibit mutations in the p53 coding sequence (6, 33, 45).

HPVs are closely associated with the development of anogenital malignancies in humans. The DNA of specific, so-called high-risk HPV types (in particular, HPV type 16 [HPV16] and HPV18) is detectable in approximately 90% of all cervical carcinomas (47). In cervical tumors, as well as in cell lines derived thereof, the E6 and E7 genes of the virus are regularly retained and expressed (36). The E6 and E7 genes of oncogenic HPVs possess transforming potential in vitro (2, 3, 30), and their expression has been shown to be required for the maintenance of the transformed and malignant phenotype of cervical cancer cells (41, 42). Interestingly, the viral gene products can form specific complexes with cellular tumor suppressor proteins. While E7 binds to the retinoblastoma gene product (7), E6 specifically interacts with the p53 protein (43), leading to a rapid degradation of p53 in vitro by a ubiquitin-dependent proteolysis pathway (5, 34). Cervical tumorigenesis might thus involve a functional inactivation of the p53 protein by either complex formation with the E6 protein of high-risk HPVs or by somatic mutations in the p53 gene. This model would provide an explanation for the inverse correlation between the presence of HPV sequences and mutations in the p53 gene.

Recent data attributed distinct biochemical functions to the wild-type p53 protein, which seem to be important for its tumor-suppressive activity. The nuclear localization, the presence of an acidic activator domain similar to those observed in transcription factors, the potential of wild-type p53 for sequence-specific DNA binding, and the transcriptional activation of genes adjacent to these binding sites suggest that p53 is involved in transcriptional control (8, 10, 11, 13, 19, 20, 29, 32, 38). Interestingly, several reports indicate that mutant p53 has lost the sequence-specific DNA binding and transcriptional transactivation function (8, 10, 11, 21, 32, 38, 39). Furthermore, specific cellular and viral oncoproteins have been implicated to inhibit p53 transactivation function (21, 27, 28, 46). It thus has been proposed that either a somatic mutation of the p53 gene or the interaction of cellular and viral oncoproteins with p53 protein can contribute to cellular transformation by interfering with p53-mediated transactivation of crucial growth-regulatory genes (40).

Little is known, however, about the DNA binding and transcriptional activity of endogenous p53 in tumor cells derived from virus-associated cancers. We therefore attempted to investigate the functional status of endogenous p53 protein in cervical cancer cells by using the HPV18-containing HeLa cell line as a model system relevant for HPV-associated carcinogenesis. If HeLa cells contained functional p53 protein, the effects of potential modulators on endogenous p53 transactivation function could be directly investigated within a human tumor cell. Under the aspect of

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the close association between certain types of HPVs and cervical tumorigenesis, it would be particularly important to investigate the effects of high-risk versus low-risk HPV E6 proteins on p53-mediated transcriptional stimulation in cervical cancer cells and to relate these findings to the activities of known cellular oncoproteins.

In the present report, we show that endogenous HeLa cell p53 protein can be detected as a sequence-specific DNA binding activity in gel retardation assays. HeLa cell p53 is transcriptionally active, as shown by its ability to transactivate p53-responsive reporter plasmids. Overexpression of wild-type p53 protein leads to a further increase in p53-mediated transcriptional stimulation, indicating that it is the amount of functional endogenous p53 which limits p53 transactivation function in HeLa cells. Cotransfection experiments show that p53 transactivation function can be efficiently repressed by high-risk HPV16 E6 but not by low-risk HPV6 E6. Similar to HPV16 E6, putative cellular oncoproteins such as human mdm-2 and mutant p53 also strongly interfered with the transactivating function of endogenous p53 in HeLa cells.

MATERIALS AND METHODS

Plasmids and oligonucleotides. Basic luciferase reporter vector pGUP.PA.8 and the p53-responsive reporter plasmid p53CON, which contains a p53-specific binding site (see oligonucleotide p53CONoli for sequence) upstream of a minimal human *hsp70* promoter, have been described in detail (13).

HPV E6 expression vectors were kindly provided by S. Sedman and J. Schiller. Briefly, plasmid p16E6 (pSDE6) contains HPV16 nucleotides 24 to 654 with a mutation in the splice-donor site at nucleotide 228, while plasmid p6E6 contains HPV6 nucleotides 34 to 672 (37). Both expression vectors contain the identical HPV16 E6 untranslated region upstream of the ATG. In both constructs, expression is controlled by the Harvey murine sarcoma virus long terminal repeat. Control vector pLTR is the same vector devoid of HPV coding sequences (37).

mdm-2 expression vectors pmdm-2S and pmdm-2AS were constructed by inserting the complete human *mdm-2* gene cDNA sequence (*Xho*I-*Bam*HI digest of plasmid mdm-2 FL4, [31]) in sense and antisense orientations, respectively, into the *Sma*I site of the human cytomegalovirus enhancer-promoter-driven expression vector pUHD 10-1 (14) by blunt-end ligation.

p53 expression vector p53wt contains the wild-type p53 coding sequence under control of the human cytomegalovirus promoter-enhancer region cloned into basic vector pLV (22), while p53/248Mut contains an arginine-to-tryptophan transversion at codon 248 of the p53 cDNA.

Double-stranded oligonucleotide p53CONoli, corresponding to a well-defined p53 binding site (13), was generated by synthesis of the complementary DNA sequences 5′-GGAC ATGCCCGGGCATGTC-3′ and 5′-GACATGCCCGGGCAT GTCC-3′ with a Gene Assembler Plus (Pharmacia), purification by polyacrylamide gel electrophoresis, and annealing. Double-stranded oligonucleotide Oct-1 (sense strand, 5′-TGTCGAATGCAAATCACTAGAA-3′) contains an Oct-1 consensus binding site (underlined) and was purchased from Promega.

Nuclear extracts and gel retardation assays. Nuclear extracts were prepared as described previously (16). For gel retardation assays, nuclear extract was mixed with 0.5 μg of poly(dA)-poly(dT) in a 25-μl reaction volume containing 10% glycerol, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 7.9), 4 mM $MgCl_2$, 30 mM KCl, 0.1 mM EDTA, 0.5 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride. After the addition of 0.1 μg of the p53-specific antibody PAb421 (Dianova) when indicated, the mixture was incubated for 5 min on ice. A total of 24,000 cpm of [γ-^{32}P]ATP end-labeled double-stranded oligonucleotide (20 to 40 fmol) was added as a probe, and incubation was continued for 15 min on ice. For competition assays, a molar excess, as specified in the text, of unlabeled double-stranded oligonucleotides was included before addition of probe DNA. DNA-protein complexes were separated from unbound probe on a 4% (29:1 cross-linking ratio) nondenaturing polyacrylamide gel and were visualized by autoradiography.

Transfections. HeLa cervical carcinoma cells, Saos-2 osteosarcoma cells, and HepG2 hepatoma cells were maintained in Dulbecco's minimal essential medium, pH 7.2, supplemented with 10% fetal calf serum. Approximately 2×10^5 cells per 6-cm dish were transfected by calcium phosphate coprecipitation as described previously (17). Transfections typically contained 3 μg of reporter plasmid and were adjusted to 6.5 μg by addition of Bluescript DNA. For cotransfections, variable amounts of expression plasmids were included as indicated in the text. For luciferase assays, cells were processed as described previously (16) and activities were quantitated with a Lumat luminometer (Berthold).

RESULTS

Detection of endogenous p53 protein in HeLa cells. The study of endogenous p53 protein in cervical carcinoma cell lines, such as HeLa, has been hampered by the extremely low abundance of p53 in these cells. The sequence-specific DNA binding activity of p53 could provide a tool to detect endogenous p53 protein in a more sensitive manner. In a recent report (13), a transcriptionally active DNA binding site for human p53 has been identified. Interestingly, p53 binding to these sequences, termed p53CON, could be strongly induced by the addition of the p53-specific monoclonal antibody PAb421 (13, 19).

Using this system, we found that exogenous p53 protein introduced into the p53-negative osteosarcoma cell line Saos-2 (25) can be specifically detected in gel retardation assays by employing oligonucleotide p53CONoli as a probe. As shown in Fig. 1A, transient transfection of an expression vector for wild-type p53 into Saos-2 cells gave rise to a prominent complex (arrow) after inclusion of PAb421 in the binding reaction (lane 4). In contrast, we did not detect p53 binding in the absence of PAb421 (lane 3), confirming that addition of this antibody strongly augments p53 binding to p53CONoli. In control experiments, no binding was observed in nuclear extracts from Saos-2 cells transfected with the same expression vector devoid of the p53 coding sequence (pLV), in either the absence or the presence of PAb421 (lanes 1 and 2, respectively). Incubation of PAb421 with p53CONoli in the absence of nuclear extract (lane 12) indicated that the antibody itself has no affinity to the oligonucleotide. In addition, p53 binding to p53CONoli is sequence specific, as increasing amounts of self-oligonucleotide, but not of a heterologous oligonucleotide containing an Oct-1 binding site, could efficiently compete for complex formation (lanes 5 to 10). Furthermore, overexpression of p53 in HeLa cells (Fig. 1A, lane 11) gave rise to a prominent complex with migration behavior identical to that of the one observed in Saos-2 cells after introduction of p53 (lane 4).

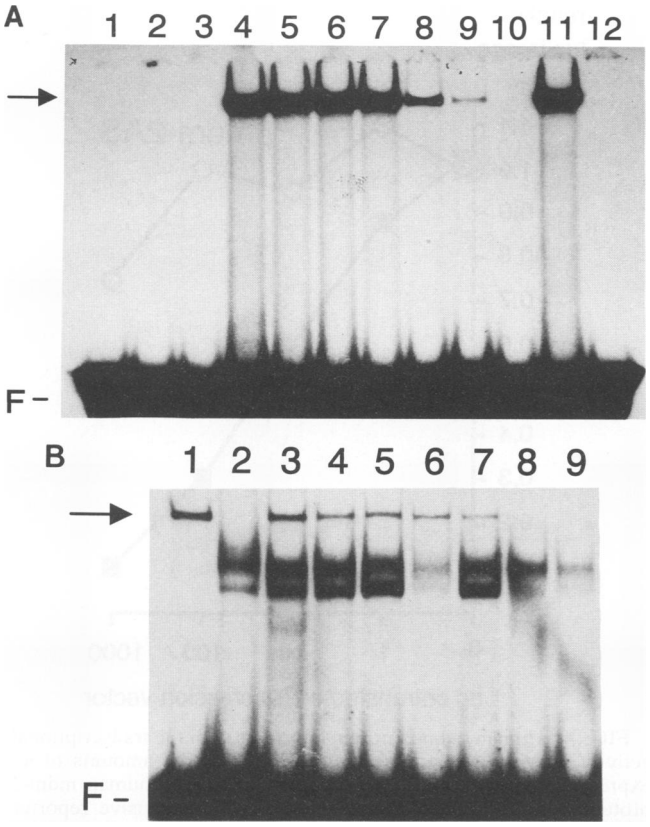

FIG. 1. Sequence-specific DNA binding of wild-type p53. (A) Induction of p53 binding activity by p53 antibody PAb421. Gel retardation analysis of 3 μg of nuclear extract after transfection of either wild-type p53 expression vector p53wt or control vector pLV, with oligonucleotide p53CONoli as a probe. Lanes 1 and 2, Saos-2 cells transfected with pLV before (lane 1) and after (lane 2) addition of p53 PAb421 to the binding reaction. Lanes 3 and 4, Saos-2 cells transfected with p53wt, without (lane 3) and with (lane 4) added PAb421. Lanes 5 to 10, as lane 4, but subjected to competition with increasing amounts (10-, 100-, and 1,000-fold molar excess) of a heterologous oligonucleotide containing an Oct-1 binding site (lanes 5 to 7, respectively) or with the same molar excesses of self-oligonucleotide p53CONoli (lanes 8 to 10, respectively). Lane 11, nuclear extract of HeLa cells after overexpression of wild-type p53 and addition of PAb421 to the binding reaction. Lane 12, incubation of PAb421 with p53CONoli in the absence of nuclear extract. (B) Detection of DNA-binding-competent endogenous p53 protein in HeLa cells. Lane 1, 0.5 μg of Saos-2 cell nuclear extract after transfection with p53wt and inclusion of PAb421 in the binding reaction. Lanes 2 and 3, 20 μg of HeLa cell nuclear extract transfected with the p53-negative control vector pLV before (lane 2) and after (lane 3) addition of PAb421. Lanes 4 to 9, as lane 3, but subjected to competition with increasing amounts of heterologous (lanes 4 to 6) or homologous (lanes 7 to 9) oligonucleotide as detailed for panel A. F, free probe.

Consistent with the absence of endogenous p53 protein in Saos-2 cells, we did not detect p53 binding in nuclear extracts of Saos-2 cells transfected with control vector pLV before or after addition of PAb421 to the binding reaction (Fig. 1A, lanes 1 and 2). In contrast, as shown in Fig. 1B, inclusion of PAb421 in the nuclear extract of control-transfected HeLa cells (lane 3) gave rise to a complex (arrow) comigrating with the p53 complex obtained after expression of wild-type p53 in Saos-2 cells (lane 1). This complex was also observed in nontransfected HeLa cells (not shown). These findings indicate that HeLa cells, devoid of exogenously introduced p53 DNA, contain DNA-binding-competent endogenous p53 protein. This conclusion was further supported in competition experiments, showing that the endogenous HeLa cell complex exhibits the same DNA binding specificity for p53CONoli (Fig. 1B, lanes 4 to 9) as wild-type p53 protein introduced into Saos-2 cells (Fig. 1A). The faster-migrating bands observed in Fig. 1B are likely to be caused by unspecific DNA binding activities, as they are eliminated by increasing amounts of both homologous and heterologous competitor oligonucleotides.

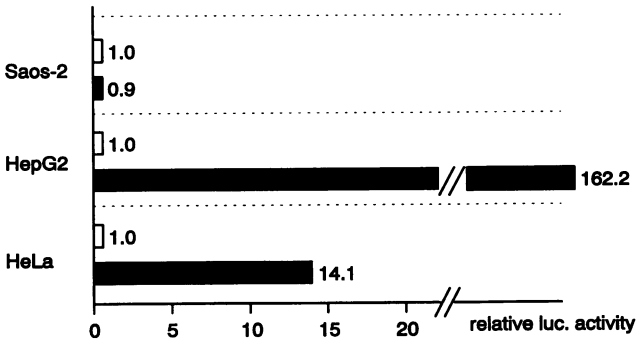

FIG. 2. Transactivation of a p53-responsive reporter plasmid by endogenous HeLa cell p53 protein. Relative luciferase activities of p53-responsive reporter plasmid p53CON (expressed as fold activation above that of the basic vector pGUP.PA.8) after transfection into Saos-2, HepG2, and HeLa cells. Results represent the average values of at least three independent transfections, each performed in triplicate. Results from individual transfections varied by less than 20%. Open boxes, pGUP.PA.8; solid boxes, p53CON.

Transactivation of p53-responsive reporter plasmids by endogenous p53. Besides its sequence-specific DNA binding capacity, p53 has been shown to possess transcriptional activation potential. The detection of DNA-binding-competent p53 in HeLa cells led us to investigate whether endogenous HeLa cell p53 can exert a transcriptional stimulatory function. We therefore analyzed the activity of a p53-responsive reporter plasmid after introduction into this cell line in comparison with Saos-2 cells and HepG2 cells.

As expected, the p53-responsive luciferase reporter plasmid p53CON (13) was not activated above the level of the basic control vector pGUP.PA.8 in Saos-2 cells, which are devoid of endogenous p53 protein (Fig. 2). In contrast, p53CON was strongly activated in HepG2 hepatoma cells (Fig. 2), which have been shown to contain unaltered levels of wild-type p53 protein (4). Consistent with the presence of endogenous p53 protein in HeLa cells, p53CON was also activated in this cell line, albeit to a lower level than in HepG2 hepatoma cells. Since this lower activity reflects the lower abundance of endogenous p53 in HeLa cells, it is possible that the HPV18 E6 in these cells acts by decreasing the level of functional p53 protein but not by inhibiting the transcriptional stimulatory activity of the p53 that remains.

We then analyzed whether augmented levels of intracellular p53 could further increase the activity of p53CON. As shown in Fig. 3, p53CON activity could be induced in Saos-2 cells, and further enhanced in HeLa cells, by the introduction of an expression vector encoding wild-type p53 protein but not by the same expression vector devoid of p53 coding sequences. These findings indicate that HeLa cells have the capacity to confer p53-mediated transactivation in response

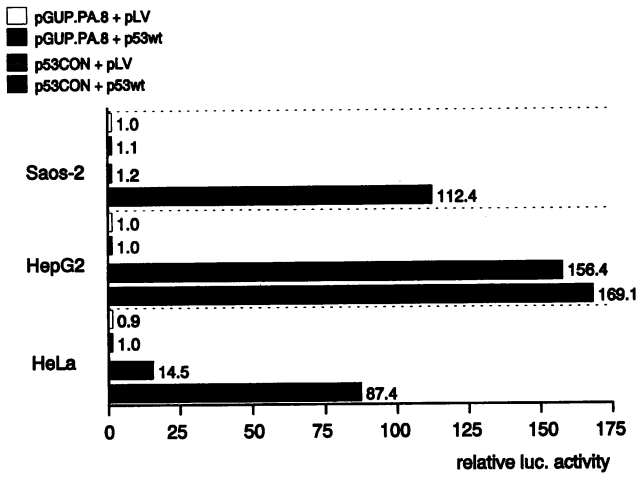

FIG. 3. Modulation of p53CON activity by augmented levels of intracellular p53. Relative luciferase activities of reporter plasmids pGUP.PA.8 and p53CON after cotransfection of 10 ng of either wild-type p53 expression vector p53wt or control vector pLV (expressed as fold activation above the respective reporter plasmid activities without cotransfected expression vector; see legend to Fig. 2). Results represent the average values from at least three independent transfections performed in triplicate, with at least two independent plasmid preparations for each construct.

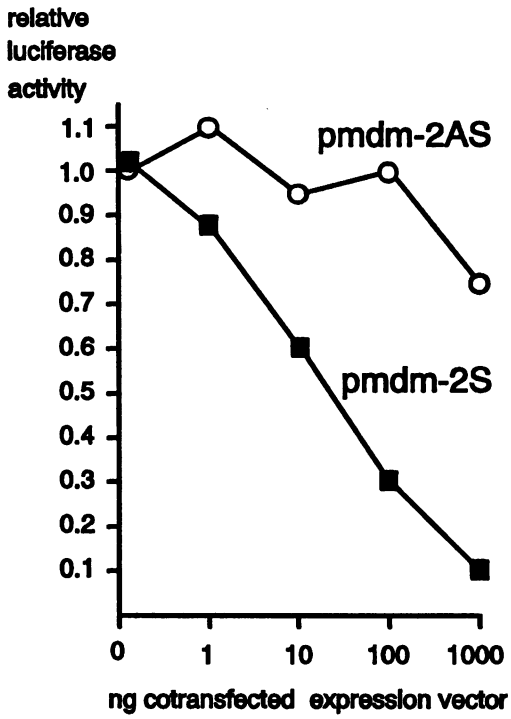

FIG. 4. Human mdm-2 protein interferes with the transcriptional activity of endogenous HeLa cell p53. Increasing amounts of an expression vector (pmdm-2S, solid boxes) for the human mdm-2 protein were cotransfected with 3 μg of p53-responsive reporter plasmid p53CON into HeLa cells. Luciferase activity is measured relative to p53CON activity without cotransfected expression plasmid (normalized to 1.0). Results obtained by cotransfection of control vector pmdm-2AS are indicated by open circles. Values are the means of at least three independent experiments with at least two independent plasmid preparations.

to varied levels of intracellular p53 and therefore that the amount of functional endogenous p53 limits p53-mediated transactivation in these cells. In contrast, p53CON was not further stimulated by the same p53 expression vector in HepG2 cells (Fig. 3) or in primary human dermal fibroblasts (not shown), most likely because of the high level of endogenous p53 activity in these cell types.

mdm-2, mutant p53, and the E6 protein of high-risk HPV16, but not of low-risk HPV6, repress transcriptional stimulation by p53. The transcriptional transactivation potential of p53 is thought to play a major role in the tumor-suppressive activity of this protein, possibly by activating cellular regulatory genes crucial for physiological growth control (40). It has been suggested that certain oncogenic proteins, such as the product of the *mdm-2* (murine double-minute 2) gene or mutant p53, contribute to cellular transformation by interfering with the transactivation function of wild-type p53 (21, 28). To examine the effect of these cellular oncoproteins on the activity of endogenous p53 and to relate these findings to the functional properties of high-risk and low-risk HPV E6 proteins, transient transfection assays were performed with HeLa cells.

A recent analysis of the murine system indicated that the mouse mdm-2 protein can form stable complexes with p53 and inhibit the transcriptional activation function of overexpressed wild-type p53 (28). To examine the effect of human mdm-2 protein on endogenous p53 transactivation function in human cells, expression vectors containing the complete human *mdm-2* coding sequence were created (see Materials and Methods). A dose-dependent repression of p53 transactivation function was observed after cotransfection of increasing amounts of the human mdm-2 expression vector (pmdm-2S) together with the p53-responsive reporter plasmid p53CON into HeLa cells (Fig. 4) but not after cotransfecting the same expression vector containing the *mdm-2* coding sequence in an inverted orientation (pmdm-2AS). In control experiments, none of the expression vectors reduced the activity of basic reporter plasmid pGUP.PA.8 (not shown).

Like mdm-2, mutant p53 has been proposed to act as an oncoprotein by forming complexes with wild-type p53 and interfering with p53-mediated transcriptional activation (21). An analysis of the effect of mutant p53 on the transactivation potential of endogenous HeLa cell p53 is indicated in Fig. 5. As shown, p53-responsive reporter plasmid p53CON was efficiently repressed by cotransfecting a construct expressing a p53 protein mutated at codon 248, but not by cotransfecting the same vector devoid of this coding sequence. A similar repression of p53CON was observed in analyzing other p53 mutants, while basic vector pGUP.PA.8 was not responsive to mutant p53 (not shown). These findings indicate that both human mdm-2 and mutant p53 can efficiently interfere with p53-mediated transactivation in HeLa cells.

To investigate whether HPV E6 proteins can interfere with the transactivation function of endogenous HeLa cell p53, we analyzed the effects of high-risk HPV16 E6 and low-risk HPV6 E6 proteins on the activity of reporter plasmid p53CON. For these studies, p53CON was cotransfected with an expression vector for either HPV16 E6 (p16E6) or HPV6 E6 (p6E6) or, as a control, with the same expression vector devoid of E6 coding sequences (pLTR). Because of the lack of antibodies which recognize both HPV16 and HPV6 E6, we cannot directly compare the level of E6 expression from both E6 expression vectors. How-

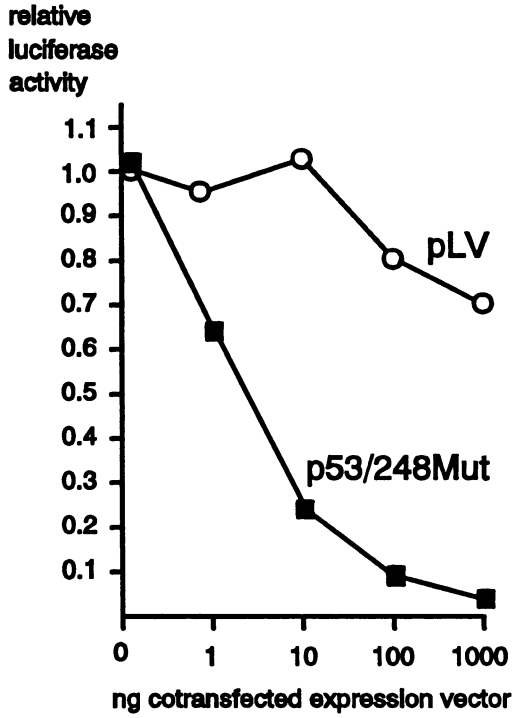

FIG. 5. Inhibition of HeLa cell p53 transactivation function by mutant p53. Various amounts of an expression vector (p53/248Mut) encoding mutant p53 protein were cotransfected together with p53CON into HeLa cells (solid boxes). Luciferase activity is measured as relative activity above p53CON activity without cotransfected expression vector (arbitrarily set at 1.0). Open circles, results from cotransfection of control vector pLV, devoid of p53 coding sequences.

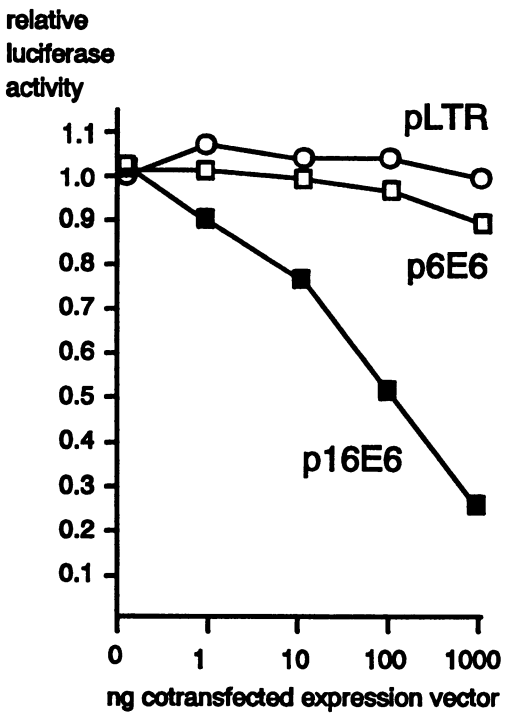

FIG. 6. Repression of endogenous p53 activity by HPV16 E6, but not by HPV6 E6, in cervical carcinoma cells. Luciferase activities obtained after cotransfection of HeLa cervical carcinoma cells with various amounts of either HPV16 E6 expression vector p16E6 (solid boxes) or HPV6 E6 expression plasmid p6E6 (open boxes) together with 3 μg of p53-responsive reporter plasmid p53CON. Results from control transfections with basic expression vector pLTR are indicated by open circles. Luciferase activities are indicated relative to p53CON activity in the absence of cotransfected expression vectors (normalized to 1.0). Results are the means of at least three independent experiments with at least two different plasmid preparations.

ever, both constructs express biologically active E6 protein, as demonstrated by their ability to transactivate the adenovirus E2 promoter (37). As shown in Fig. 6, increasing amounts of cotransfected HPV16 E6 expression vector (p16E6) efficiently repressed p53CON activity, indicating an interference with the transcriptional activation function of endogenous p53 by HPV16 E6. In contrast, the activity of p53CON was not influenced by the same expression vector devoid of the E6 gene (pLTR), demonstrating that the repression of p53 transactivation function is dependent on HPV16 E6 coding sequences. Moreover, expression of the E6 protein from the low-risk-type HPV6 (p6E6) did not interfere with p53-mediated transcriptional stimulation (Fig. 6). The same type of repression was observed in analyzing the influence of the E6 expression vectors on p53-mediated transcriptional stimulation in primary human dermal fibroblasts (not shown), indicating that the effects of the viral proteins are independent of endogenous HPV sequences. The activity of basic luciferase vector pGUP.PA.8 was not repressed by the E6 expression vectors (not shown). These findings indicate that HPV16 E6, but not HPV6 E6, can efficiently interfere with p53-mediated transactivation in HeLa cervical carcinoma cells.

DISCUSSION

The detection of functionally active p53 protein in HeLa cells allowed us to investigate directly within a cervical cancer cell the in vivo effects of putative modulators on endogenous p53 function. Despite the presence of translatable mRNA (26), previous attempts to detect p53 protein in this cell line have been controversial. In some reports, p53 could not be detected in Western blotting (immunoblotting) experiments (1, 44), while others did obtain weak signals in analogous experiments (23, 33). By employing gel retardation assays, we could significantly improve the sensitivity of p53 detection. In these experiments, we took advantage of the observation that the monoclonal antibody PAb421 strongly induces the binding of p53 to DNA (13), probably by altering the conformation of the C terminus of p53 (19). Our results further demonstrate that endogenous HeLa cell p53 is competent for sequence-specific DNA binding and significant transactivation of a p53-responsive reporter plasmid, strongly suggesting that it is wild type.

The results obtained in this study indicate that in HeLa cells not all of the intracellular p53 protein is functionally inactivated by high-risk E6 transcribed from integrated viral sequences, possibly because of the very low level of E6 expression in this cell line (35). This conclusion is supported by the observation that p53 transactivation function could be further repressed in HeLa cells by augmenting the level of intracellular high-risk E6 protein by transient overexpression of exogenously introduced HPV16 E6. Since high-risk E6 protein can induce an efficient degradation of p53 in vitro

(34), the modulation of p53-mediated transactivation by HPV16 E6 could indicate that the p53 protein level limits its transcriptional stimulatory activity in HeLa cells. However, as the degradation of p53 by high-risk E6 protein seems to be less pronounced in vivo than in vitro (18, 23, 33), one must also consider the possibility that in vivo the degradation of p53 is not the sole factor leading to the repression of p53-mediated transactivation. For example, E6 might titrate out functional p53 by the formation of transcriptionally inactive protein-protein complexes. More work is required to distinguish these possibilities.

From our results, it could be inferred that the tumorigenic phenotype of a cervical cancer cell does not require the complete inactivation of p53-mediated transcriptional stimulation. This suggests the existence of a threshold level for p53 activity below which p53 cannot efficiently exert its normal tumor-suppressive function. Alternatively, the tumorigenic phenotype might require some other activity of p53 that is completely abolished by E6. We believe that the former possibility is more likely, since the transcriptional stimulation of important cellular regulatory genes by p53 has been implicated to play a key role for its function as a tumor suppressor protein and mutant forms of p53 devoid of tumor-suppressive activity generally have lost the ability to transactivate p53-responsive reporter plasmids (8, 10, 11, 21). Thus, in cervical carcinogenesis, the inactivation of p53 transactivation function by the E6 protein of high-risk HPV16 would result in the same biological effect as a somatic mutation in the p53 gene. This could explain why cervical cancer cells usually either carry alterations in their p53 genes or contain high-risk HPV E6 sequences.

After completion of this work, Mietz et al. (27) published a study investigating the effect of high-risk versus low-risk HPV E6 proteins on p53-mediated transcriptional stimulation by performing cotransfection assays with expression vectors for wild-type p53 and HPV E6 proteins in p53-negative lung cancer cells. Consistent with our results concerning the effect of HPV E6 proteins on endogenous p53 transactivation function in HeLa cervical carcinoma cells, an interference with p53-mediated transcriptional stimulation was observed for high-risk HPV16 E6 but not for low-risk HPV11 E6. The finding that HPV6 and HPV11 E6 do not exert an inhibitory function on p53-mediated transcriptional stimulation correlates well with the observation that the E6 protein of low-risk HPV types does not have immortalizing and transforming activities. Intriguingly, corresponding results have been recently obtained in an analysis of another DNA tumor virus oncogene (46). In this report, a strong correlation has been observed between the ability of adenovirus E1B proteins to inhibit p53-mediated transactivation and their potential to transform primary cells in cooperation with E1A. These results experimentally support the notion of common mechanisms of cell transformation by different DNA tumor viruses.

In addition to viral gene products, cellular proteins also have been implicated in the inhibition of p53-mediated transactivation. Consistent with recent reports demonstrating that overexpressed mutant p53 can interfere with the transcriptional activation of reporter plasmids by cotransfected wild-type p53 (21, 32, 39), we observed in our system a repression of the transactivation function of endogenous wild-type p53 by mutant p53. A second cellular protein, namely, the product of the *mdm-2* (murine double-minute 2) gene, recently has been shown to be able to interfere with the transactivation function of wild-type p53 in murine cells (28). Here we show that also the product of the human *mdm-2* gene, which is amplified in a large number of sarcomas (31), can interfere efficiently with p53-mediated transcriptional stimulation in human cells. Interestingly, analogous to the situation in E6-containing cervical carcinomas, human sarcomas containing amplified *mdm-2* sequences usually harbor wild-type p53 alleles (31).

Both mutant p53 and the human mdm-2 protein may act as oncoproteins by inhibiting endogenous wild-type p53 function in a dominant negative fashion (40). The results obtained in this study indicate that, by interfering with the transcriptional stimulatory function of wild-type p53, the E6 gene product of HPV16 can act in a manner similar to that of these putative cellular oncoproteins. Furthermore, since HPV6 E6 does not have this activity, the repression of p53 transactivation might be one factor contributing to the different transforming potentials of high-risk versus low-risk HPV types.

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