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The Human Papillomavirus E7 Oncoprotein and the Cellular Transcription Factor E2F Bind to Separate Sites on the Retinoblastoma Tumor Suppressor Protein

ERICA W. WU, KAREN E. CLEMENS, DONALD V. HECK, AND KARL MÜNGER*

Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, Maryland 20892

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The ability of the high-risk and low-risk human papillomavirus E7 oncoproteins to disrupt complexes of the retinoblastoma tumor suppressor protein pRB and the cellular transcription factor E2F was studied. The ability of E7 to disrupt this transcription factor complex correlated with the different pRB binding efficiencies of the high-risk and low-risk human papillomavirus-encoded E7 proteins. The pRB binding site was the sole determinant for these observed differences. The phosphorylation status of the casein kinase II site that is immediately adjacent to the pRB binding site in E7 had no marked effect on this biochemical property of E7. Peptides consisting of the pRB binding site of E7, however, were not able to disrupt the pRB/E2F complex. These data suggest that additional carboxy-terminal sequences in E7 are also required for the efficient disruption of the pRB/E2F complex and that E7 and E2F may interact with nonidentical sites of pRB.

The human papillomaviruses (HPVs) associated with lesions of the anogenital tract can be divided into two groups: the low-risk HPVs, such as HPV-6 and HPV-11, that are associated with benign lesions such as condyloma acuminata and the high-risk HPVs that are associated with cervical intraepithelial neoplasia, which can progress to cervical carcinoma (40). The E7 genes of the anogenital tract-associated HPVs encode multifunctional proteins that are structurally and functionally related to the adenovirus E1A proteins (Ad E1A) and the simian virus 40 large tumor antigen (SV40 TAg) (13, 30, 37). The E7 proteins encoded by the high-risk HPVs differ from the E7 proteins encoded by the low-risk HPVs in their in vitro transformation potential as well as in a number of biochemical and biological properties. The high-risk HPV E7 proteins efficiently cooperate with an activated *ras* oncoprotein to transform baby rat kidney cells (26, 36, 37) and together with E6 are necessary for the efficient immortalization of primary human epithelial cells (16, 24). The high-risk HPV-encoded E7 proteins form complexes with pRB with higher binding efficiency than the E7 proteins encoded by the low-risk HPVs (3, 15, 25). An intact pRB binding site of the HPV E7 protein was found to be necessary for *ras* cooperation (29, 38), and the differences in transformation efficiencies of the high-risk and low-risk HPV-encoded E7 proteins closely correlate with their respective pRB binding efficiencies (3, 26). Indeed, it was found that the E7 proteins encoded by the low-risk and high-risk HPVs contain a single consistent amino acid sequence difference in their pRB binding sites (aspartic acid 21 in HPV-16 E7 versus glycine 22 in HPV-6 E7) that largely accounts for the differences in *ras* cooperation potential and pRB binding efficiency of the low-risk and high-risk HPV-encoded E7 proteins (17). Both low-risk and high-risk HPV E7 proteins efficiently transactivate the Ad E2 promoter (26, 36, 39). An intact pRB binding site on HPV E7 is required for transactivation of the Ad E2 promoter (12, 29, 38, 39). Similar to the 12S form of Ad E1A, HPV E7 requires intact E2F binding sites in the Ad E2 promoter for transactivation of this promoter (28). It has also been shown that E7, like 12S Ad E1A and SV40 TAg, is able to disrupt heteromolecular complexes of the cellular transcription factor E2F (1, 7, 28). As measured by electrophoretic mobility shift assays (EMSAs), E2F bound to DNA is present in at least two distinguishable heteromolecular complexes in normal cells. One of these complexes contains pRB (2, 5, 6, 8, 34), and the other contains the pRB-related protein p107 as well as cyclin A and cdk2 (5, 10, 22, 27, 34). The HPV-16 E7 protein was shown to dissociate the pRB-containing E2F-DNA complex more efficiently than the p107/cyclin A/cdk2-containing E2F-DNA complex (7). The pRB/E2F complex was also found to be absent or diminished in a panel of HPV-positive and HPV-negative human cervical carcinoma cell lines, suggesting that the loss of this interaction may be a step in cervical carcinogenesis (7). Here it is shown that HPV-16 E7 is able to disrupt the pRB/E2F complex with at least a 10-fold-higher efficiency than HPV-6 E7. This difference between HPV-6 and HPV-16 E7 maps entirely to the pRB binding site of E7 and correlates with the difference in pRB binding potential that was previously reported for the two HPV E7 proteins (3, 17, 25). It is also shown that the E7 pRB binding site, although necessary for the disruption of the heteromolecular E2F complexes, is not sufficient for this activity, implying that E7 and E2F binding sites on pRB are not identical.

It was previously shown that purified bacterial fusion proteins of HPV-16 E7 with *Schistosoma japonicum* glutathione *S*-transferase (GST-16 E7) can efficiently disrupt heteromolecular E2F complexes and that this property of GST-16 E7 required an intact pRB binding site on E7 (7, 28). Similar results were obtained here when these experiments were performed with nuclear extracts from the human myeloid leukemia cell line ML-1 and with a short double-stranded E2F oligodeoxynucleotide (5) as a probe (Fig. 1A and B). The presence of pRB and cyclin A in the corresponding complexes was confirmed by supershift experiments with the pRB-specific monoclonal antibodies C36 and PMG3-245.11 and the cyclin A-specific monoclonal antibody C160 (all from PharMingen) as previously described (5, 34; data not shown). Titration of GST-16 E7 into a standard E2F

* Corresponding author.

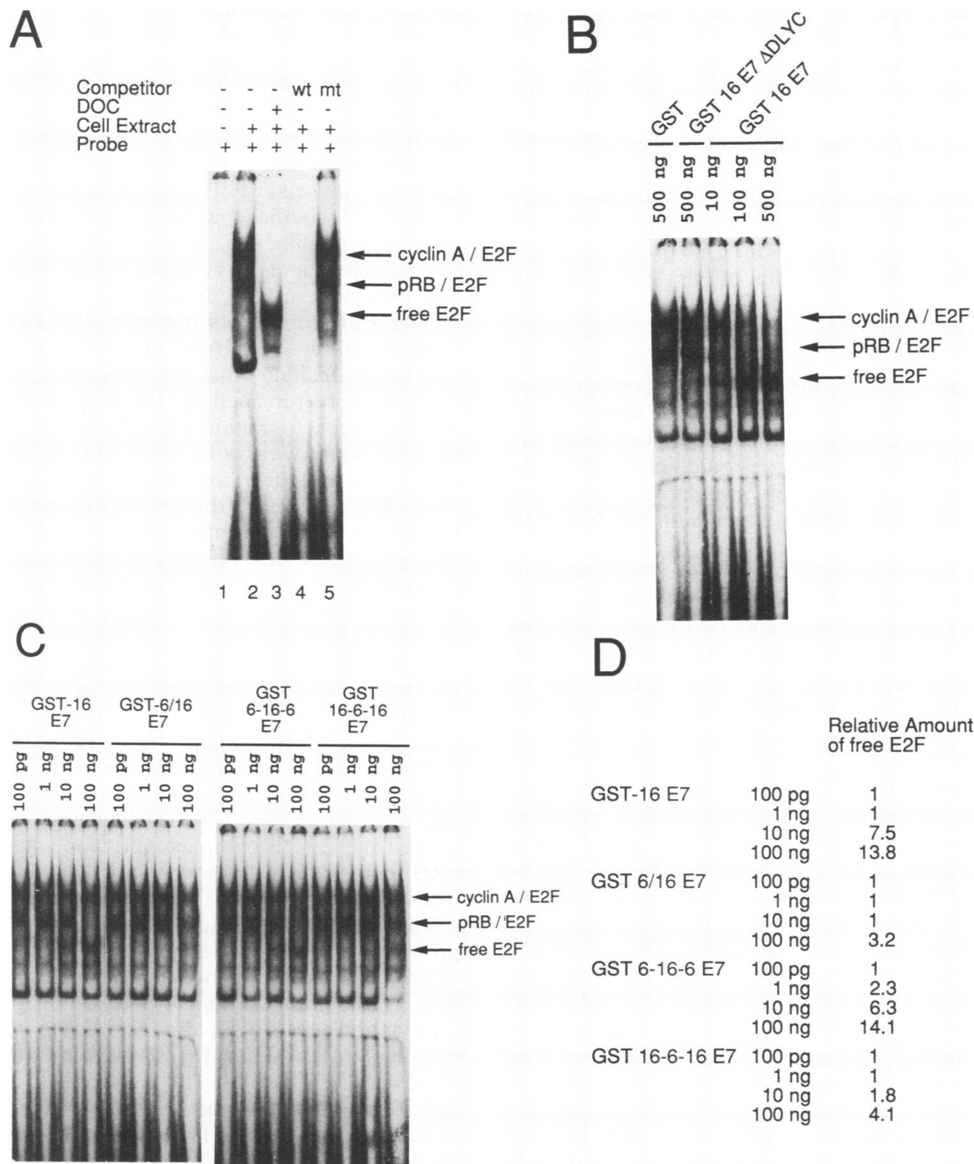

FIG. 1. (A) One nanogram of a double-stranded ^{32}P-labeled oligodeoxynucleotide probe (5), ATTTAAGTTTCGCGCCCTTTCTCAAT TAA (lane 1), was used and reacted with 10 μg of nuclear extract (33) of the human myeloid leukemia cell line ML-1 (lane 2). To disrupt protein-protein complexes, the nuclear extracts were treated with 0.16% deoxycholate (DOC) for 10 min at 25°C (lane 3). The specificity of the bands was assessed by competition experiments with a 100-fold molar excess of either the wild-type (wt) E2F oligodeoxynucleotide (see above) (lane 4) or a mutant (mt) oligodeoxynucleotide, ATTTAAGTTTCGATCCCTTTCTCAATTAA (lane 5). EMSA reactions were carried out in a volume of 25 μl in 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-KOH (pH 7.6)–0.1 mM EDTA–1 mM $MgCl_2$–40 mM KCl–10% (vol/vol) glycerol–0.1% (vol/vol) Nonidet P-40–1 μg of salmon sperm DNA at 25°C (5). Products were resolved on nondenaturing 4% polyacrylamide gels in 22.5 mM Tris-borate–0.5 mM EDTA at 4°C at a constant voltage (14 V/cm). The positions of free E2F as well as the pRB/E2F and cyclin A/E2F complexes are indicated. The presence of these proteins in the corresponding complexes was confirmed by the use of antibodies as described in the text. (B) Effects of HPV E7 on E2F EMSAs. A total of 500 ng of unfused *S. japonicum* glutathione *S*-transferase (GST) (lane 1), the same amount of a GST fusion protein with the pRB binding-negative HPV-16 E7 ΔDLYC mutant (lane 2), or various amounts of a GST fusion protein with wild-type HPV-16 E7 (GST-16 E7) (lanes 3 to 5) were added to a standard E2F EMSA. The construction of these GST fusion proteins by using the plasmid pGex 2T (Pharmacia) has been described (28). GST fusion proteins were expressed in *Escherichia coli* HB101 and purified by affinity chromatography on glutathione Sepharose 4B (Pharmacia) as previously described (28). Protein concentrations were measured by using the Bio-Rad assay, and the proteins were >80% pure as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Coomassie blue staining. (C) The ability of E7 to disrupt heteromolecular E2F complexes correlates with the pRB binding efficiency of the E7 protein. A total of 0.1 to 100 ng each of GST-16 E7, GST-6/16 E7, GST 6-16-6 E7, and GST 16-6-16 E7 was titrated into standard E2F EMSAs as indicated. The data in the different panels of this figure are all derived from a single experiment. The construction of these GST E7 proteins has been described (17). The proteins were expressed, purified, and analyzed as described previously (28). (D) Densitometric quantitation of the levels of free E2F shown in panel C performed with a model P.D. laser densitometer and ImageQuant software (Molecular Dynamics, Sunnyvale, Calif.).

EMSA leads to the gradual disruption of the heteromolecular E2F complexes as manifested by the accumulation of free E2F (Fig. 1B). At 100 ng of GST-16 E7, the pRB/E2F complex is completely disrupted (Fig. 1B). The cdk2/cyclin A/p107-E2F complex is only slightly diminished but is disrupted at higher amounts of GST-16 E7 (Fig. 1B, lane 5). Addition of up to 1 μg of either unfused GST or GST-16 E7 ΔDLYC that contains a disrupted pRB binding site had no effect on the gel shift patterns (7) (Fig. 1B). A fusion of GST with a chimeric HPV-6/HPV-16 E7 protein (GST-6/16 E7) was also tested in a similar experiment. This protein consists of the amino-terminal 48 amino acids of HPV-6 E7 fused to amino acids 49 to 98 of HPV-16 E7 (26). It was previously shown that the carboxy-terminal sequences of the high-risk and low-risk HPV-encoded E7 proteins are interchangeable and do not markedly influence the transforming or transcriptional transactivating properties of E7 (26). The GST-6/16 E7 fusion protein did not efficiently function in disrupting the heteromolecular E2F-DNA complexes, and only at the highest concentration of this protein were a slight decrease of the E2F complexes and concomitant accumulation of free E2F observed (Fig. 1C and D).

Similar results were obtained with several independent preparations of the GST-16 E7 and GST-6/16 E7 proteins (compare Fig. 1B and C) and with nuclear extracts from the human histiocytic lymphoma cell line U-937 as well as with E2F oligodeoxynucleotide probes derived from the *c-myc* or dihydrofolate reductase promoters (19, 23) or the C9 oligodeoxynucleotide probe described by Chittenden et al. (8) (data not shown).

To assess the role of the pRB binding site for the observed differences in the activities of the two fusion proteins, chimeric E7 proteins that contain the HPV-6 E7 pRB binding site in the background of HPV-16 E7 (GST 16-6-16 E7) or with the HPV-16 E7 pRB binding site in the background of the 6/16 chimeric E7 protein (GST 6-16-6 E7) were tested for their ability to disrupt the heteromolecular E2F complexes as measured by E2F EMSA. As shown in Fig. 1C and D, the GST 6-16-6 E7 protein was able to disrupt the pRB/E2F complex as efficiently as the GST-16 E7 protein, whereas the GST 16-6-16 E7 protein, like the GST-6/16 E7 protein, was much less efficient in these assays. Therefore, the observed differences between GST-6/16 E7 and GST-16 E7 in disrupting the E2F complexes are due solely to the pRB binding sites. These results parallel the previously reported pRB binding efficiencies of these same chimeric E7 proteins in that an exchange of the pRB binding site between the HPV-6 and HPV-16 E7 proteins led to a 7- to 10-fold difference in the efficiency of pRB binding (17). Surprisingly, these chimeric proteins did not markedly differ in their ability to transactivate the Ad E2 promoter (17). One explanation of this could be that the levels of E7 proteins produced by the transient cotransfection assays are high enough to titrate out an amount of E2F sufficient to result in the transcriptional activation of the E2F responsive reporter gene. Moreover, while it was clearly demonstrated that HPV-16 E7, like the 12S form of Ad E1A, required the integrity of the E2F sites in the Ad E2 promoter for the transcriptional transactivation of the Ad E2 promoter, it cannot be ruled out that additional *cis* elements may also be involved in mediating the transactivation property of HPV E7. Indeed, mutation of the ATF site in the Ad E2 promoter led to a two- to fourfold decrease in transactivation both by the 12S form of Ad E1A and by HPV-16 E7 (28). Experiments with additional E7-responsive promoters may be necessary in order to delineate the *cis* requirements for the HPV E7 transcriptional transactivation property in more detail.

Previous mutational analyses of the CK II phosphorylation site, which is located immediately adjacent to the pRB binding site in HPV E7 (3, 14), had indicated that phosphorylation-negative E7 mutants were slightly impaired for Ad E2 transactivation (12, 29) and that the high-risk HPV E7 proteins were better substrates for CK II phosphorylation than the low-risk HPV E7 proteins (3). Therefore, the question of whether the phosphorylation status of the CK II site had an influence on the ability of E7 to disrupt the heteromolecular E2F complexes was tested. No difference was observed in the efficiency of disrupting the heteromolecular E2F complexes between the GST 16 E7 wild-type protein and the GST-16 E7 D-31–D-32 protein, in which the two serine residues that are substrates for CK II phosphorylation were replaced by the negatively charged aspartic acid residues to mimic phosphoserine residues (data not shown). Moreover, both the wild-type form of GST 16 E7 and the CK II phosphorylation-negative mutant GST-16 E7 A-31–A-32, in which the two serine residues that are substrates for CK II are replaced by two alanine residues, were subjected to in vitro phosphorylation by CK II as described previously (17) and then titrated into standard E2F EMSAs. Both proteins retained the ability to disrupt the heteromolecular E2F complexes at similar concentrations (data not shown). These data indicate that phosphorylation of HPV-16 E7 by CK II does not markedly alter the ability of E7 to disrupt the heteromolecular E2F complexes.

After the pRB binding site of HPV E7 was mapped as being necessary for the disruption of the pRB/E2F complex (7) (Fig. 1B) and after it was established that the pRB binding site is a determinant for the efficiency of the E7 protein to disrupt these E2F complexes, it was asked whether the E7 pRB binding site alone was sufficient for the disruption of these E2F complexes. To be able to directly compare these results with those obtained with GST full-length E7 fusion proteins, GST E7 peptide fusion proteins were constructed. These encoded one or two copies of amino acids (aa) 1 to 35 (CR 1 homology domain – pRB binding site–CK II phosphorylation site) as well as aa $(1 \text{ to } 35)_2$ΔDLYC (pRB binding negative) of the HPV-16 E7 protein. Titration of each of these GST E7 peptide fusion proteins into standard E2F EMSAs had no effect on the shift pattern (Fig. 2A). Similar results were obtained with a synthetic peptide encompassing aa 2 to 32 of the HPV-16 E7 sequence that had previously been shown to bind to pRB and p107 (11) (data not shown). To determine whether the GST-16 E7 peptide fusion proteins that contained an intact pRB binding site could still bind to the pRB/E2F complexes, these peptide fusion proteins were tested for their ability to block the action of GST-16 E7 on the E2F transcription factor complexes. Preincubation of 500 ng of GST-16 (1–35) E7 but not GST-16 (1–35 ΔDLYC) E7 could efficiently block the ability of wild-type GST-16 E7 to disrupt the heteromolecular E2F complexes (Fig. 2B). These experiments showed that the GST-16 E7 peptide fusion proteins were still able to efficiently bind to pRB (and p107) but that they were inactive in disrupting the corresponding E2F complexes. This strongly suggests that E2F and E7 bind to separate sites on pRB (and p107).

Interestingly, a recent report showed that a fragment of pRB consisting only of the binding pocket (aa 379 to 792), which is necessary and sufficient for binding to Ad E1A and SV40 TAg, was not sufficient for growth suppression in human RB-/- cells or for binding to E2F as measured by EMSA (31). Amino acid sequences at the carboxy terminus

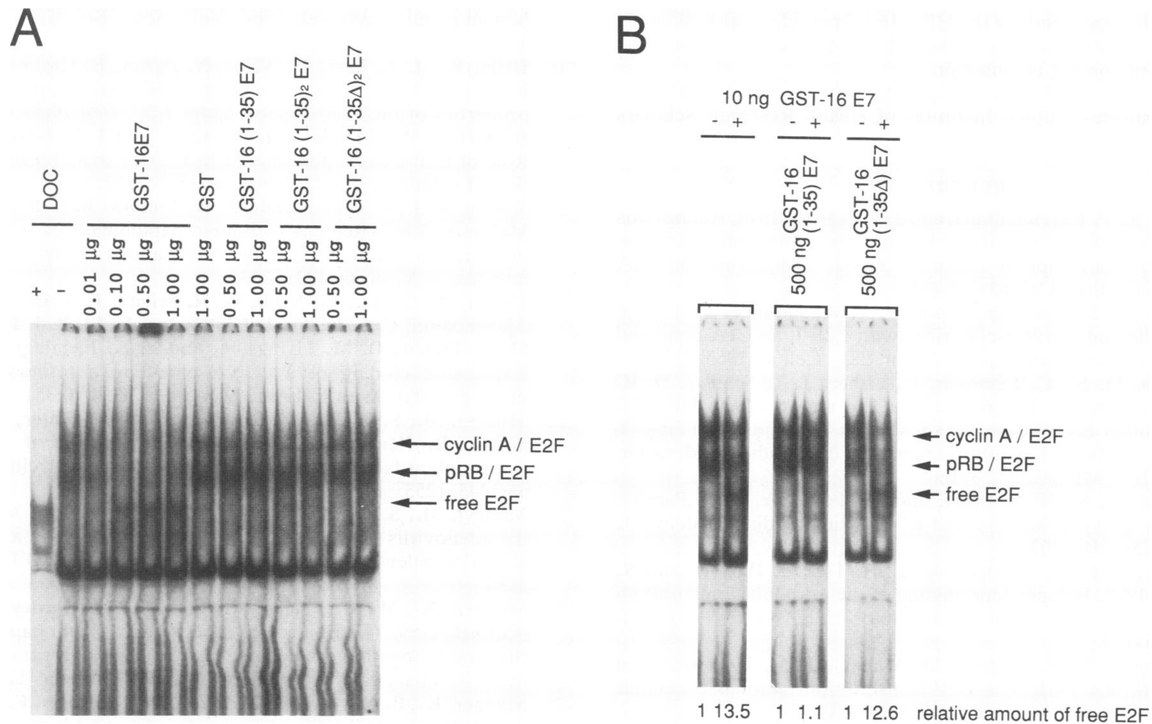

FIG. 2. Amino acid sequences in HPV-16 E7 other than the pRB binding site are necessary for the disruption of the E2F transcription factor complexes. (A) GST peptide fusion proteins consisting of the HPV-16 E7 pRB binding site are not able to disrupt heteromolecular E2F transcription factor complexes. A total of 0.1 to 500 ng of GST 16-E7 and 500 ng and 1 µg of each GST-16 E7 peptide fusion protein encoding one copy [GST-16 (1–35) E7] or two copies [GST-16 $(1–35)_2$ E7] of aa 1 to 35 or two copies of aa 1 to 35 with a 4-aa deletion, ΔDLYC, in the pRB binding site [GST $(1–35\Delta)_2$ E7] were added to standard E2F EMSAs. GST fusion proteins were expressed and purified as described above. (B) GST peptide fusion proteins consisting of the HPV-16 E7 pRB binding site can prevent the disruption of heteromolecular E2F complexes by GST-16 E7. A total of 500 ng of GST E7 peptide fusion proteins encoding aa 1 to 35 [GST-16 (1–35) E7] or aa 1 to 35 with a 4-aa deletion in the pRB binding site [GST-16 (1–35Δ) E7] was added to E2F EMSAs and tested for the ability to block the action of 10 ng of GST-16 E7 on the E2F transcription factor complexes. The level of free E2F in each lane was estimated by densitometric scanning as described in the legend to Fig. 1D and is indicated. The data in all panels of this figure are derived from a single experiment.

of pRB (aa 792 and 928) that are located outside the binding pocket were found to be also required for both the growth-suppressive activity in RB-/- cells and the E2F binding property (31). In addition, the sequence of the recently cloned E2F-1 transcription factor does not contain the pRB binding core consensus sequence L-X-C-X-E that is contained within the pRB binding sites of the Ad E1A, HPV E7, and SV40 TAg (18, 21) and that was detected in other pRB-associated cellular proteins (9, 21). This further supports the idea that E2F binds to sites on pRB and p107 different from those used by the viral oncoproteins and the L-X-C-X-E-containing cellular proteins.

These data also suggest that additional amino acid sequences in E7 located between aa 35 and 98 may be required for the full activity of HPV E7 to disrupt heteromolecular E2F-DNA complexes. These sequences, however, do not constitute a second independent binding site for pRB on the E7 protein, since deletion of the pRB binding site (ΔDLYC) in the context of the full-length E7 protein abrogates the ability of E7 to form a complex with pRB (25) and to disrupt the E2F/pRB complexes bound to DNA (7) (Fig. 1B). Similarly, the pRB binding-negative E7 mutant (ΔDLYC) is also inactive in transactivating the Ad E2 promoter (29).

So far, most mutagenic analyses of E7 have failed to map any specific activities to the carboxy-terminal portions of the E7 molecule. Indeed, most of these studies suggest that the carboxy-terminal portion of E7 which contains two copies of the C-X-X-C motif required for zinc binding (4) may be necessary for protein stability and/or subcellular localization (12, 29, 38, 39). It was recently reported that the pRB binding activity of HPV-16 E7 is not required for the HPV-16 E6/E7-induced immortalization of primary human foreskin keratinocytes (20). The only HPV-16 E7 mutant that scored negative in the keratinocyte immortalization assay contained a point mutation at the second C-X-X-C motif at amino acid residue 91 (cysteine to glycine) (20). Interestingly, a recent study by Stirdivant et al. (35) reported that pRB binding was necessary but not sufficient for HPV-16 E7 to abrogate the ability of pRB to bind to DNA cellulose. Amino acid sequences in the carboxy terminus between residues 60 and 75 in E7 were shown to be important for this activity. Finally, a study by Rawls et al. (32) suggested that an HPV-16 E7 peptide encompassing amino acid residues 67 to 98 was able to transactivate the Ad E2 promoter when the corresponding peptides were microinjected together with a reporter plasmid into HeLa cells.

In conclusion, the data presented suggest that amino acid sequences at the carboxy terminus of the HPV E7 proteins are important for HPV E7's function and may somehow modulate E2F activity or the activity of a factor in the E2F pathway.

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REFERENCES

1. **Bagchi, S., P. Raychaudhuri, and J. R. Nevins.** 1990. Adenovirus E1A proteins can dissociate heteromeric complexes involving the E2F transcription factor: a novel mechanism for E1A trans-activation. Cell **62:**659–669.
2. **Bagchi, S., R. Weinmann, and P. Raychaudhury.** 1991. The retinoblastoma protein copurifies with E2F-I, an E1A-regulated inhibitor of the transcription factor E2F. Cell **65:**1063–1072.
3. **Barbosa, M. S., C. Edmonds, C. Fisher, J. T. Schiller, D. R. Lowy, and K. H. Vousden.** 1990. The region of the HPV E7 oncoprotein homologous to adenovirus E1a and SV40 large T antigen contains separate domains for Rb binding and casein kinase II. EMBO J. **9:**153–160.
4. **Barbosa, M. S., D. R. Lowy, and J. T. Schiller.** 1989. Papillomavirus polypeptides E6 and E7 are zinc-binding proteins. J. Virol. **63:**1404–1407.
5. **Cao, L., B. Faha, M. Dembski, L.-H. Tsai, E. Harlow, and N. Dyson.** 1992. Independent binding of the retinoblastoma protein and p107 to the transcription factor E2F. Nature (London) **355:**176–179.
6. **Chellappan, S., S. Hiebert, M. Mudryj, J. M. Horowitz, and J. R. Nevins.** 1991. The E2F transcription factor is a cellular target for the RB protein. Cell **65:**1053–1061.
7. **Chellappan, S., V. B. Kraus, B. Kroger, K. Münger, P. M. Howley, W. C. Phelps, and J. R. Nevins.** 1992. Adenovirus E1A, simian virus 40 tumor antigen, and human papillomavirus E7 protein share the capacity to disrupt the interaction between the transcription factor E2F and the retinoblastoma gene product. Proc. Natl. Acad. Sci. USA **89:**4549–4553.
8. **Chittenden, T., D. M. Livingston, and W. G. Kaelin, Jr.** 1991. The T/E1A-binding domain of the retinoblastoma protein can interact selectively with a sequence-specific DNA-binding protein. Cell **65:**1073–1082.
9. **Defeo-Jones, D., P. S. Huang, R. E. Jones, K. M. Haskell, G. A. Vuocolo, M. G. Hanobik, H. E. Huber, and A. Oliff.** 1991. Cloning of cDNAs for cellular proteins that bind to the retinoblastoma gene product. Nature (London) **352:**251–254.
10. **Devoto, S. H., M. Mudryj, J. Pines, T. Hunter, and J. R. Nevins.** 1992. A cyclin A-protein kinase complex possesses sequence-specific DNA binding activity: $p33^{cdk2}$ is a component of the E2F-cyclin A complex. Cell **68:**167–176.
11. **Dyson, N., P. Guida, K. Münger, and E. Harlow.** 1992. Homologous sequences in adenovirus E1A and human papillomavirus E7 proteins mediate interaction with the same set of cellular proteins. J. Virol. **66:**6893–6902.
12. **Edmonds, C., and K. H. Vousden.** 1989. A point mutational analysis of human papillomavirus type 16 E7 protein. J. Virol. **63:**2650–2656.
13. **Figge, J., T. Webster, T. F. Smith, and E. Paucha.** 1988. Prediction of similar transforming regions in simian virus 40 large T, adenovirus E1A, and *myc* oncoproteins. J. Virol. **62:**1814–1818.
14. **Firzlaff, J. M., D. A. Galloway, R. N. Eisenman, and B. Lüscher.** 1989. The E7 protein of human papillomavirus type 16 is phosphorylated by casein kinase II. New Biol. **1:**44–53.
15. **Gage, J. R., C. Meyers, and F. O. Wettstein.** 1990. The E7 proteins of the nononcogenic human papillomavirus type 6b (HPV-6b) and of the oncogenic HPV-16 differ in retinoblastoma protein binding and other properties. J. Virol. **64:**723–730.
16. **Hawley-Nelson, P., K. H. Vousden, N. L. Hubbert, D. R. Lowy, and J. T. Schiller.** 1989. HPV16 E6 and E7 proteins cooperate to immortalize human foreskin keratinocytes. EMBO J. **8:**3905–3910.
17. **Heck, D. V., C. L. Yee, P. M. Howley, and K. Münger.** 1992. Efficiency of binding to the retinoblastoma protein correlates with the transforming capacity of the E7 oncoproteins of the human papillomaviruses. Proc. Natl. Acad. Sci. USA **89:**4442–4446.
18. **Helin, K., J. A. Lees, M. Vidal, N. Dyson, E. Harlow, and A. Fattaey.** 1992. A cDNA encoding a pRB-binding protein with properties of the transcription factor E2F. Cell **70:**337–350.
19. **Hiebert, S. W., M. Blake, J. Azizkhan, and J. R. Nevins.** 1991. Role of E2F transcription factor in E1A-mediated *trans* activation of cellular genes. J. Virol. **65:**3547–3552.
20. **Jewers, R. J., P. Hildebrandt, J. W. Ludlow, B. Kell, and D. J. McCance.** 1992. Regions of human papillomavirus type 16 E7 oncoprotein required for immortalization of human keratinocytes. J. Virol. **66:**1329–1335.
21. **Kaelin, W. G., Jr., W. Krek, W. R. Sellers, J. A. DeCaprio, F. Ajchenbaum, C. S. Fuchs, T. Chittenden, Y. Li, P. J. Farnham, M. A. Blanar, D. M. Livingston, and E. K. Flemington.** 1992. Expression cloning of a cDNA encoding a retinoblastoma-binding protein with E2F-like properties. Cell **70:**351–364.
22. **Mudryj, M., S. H. Devoto, S. W. Hiebert, T. Hunter, J. Pines, and J. R. Nevins.** 1991. Cell cycle regulation of the E2F transcription factor involves an interaction with cyclin A. Cell **65:**1243–1253.
23. **Mudryj, M., S. W. Hiebert, and J. R. Nevins.** 1990. A role for the adenovirus inducible E2F transcription factor in a proliferation dependent signal transduction pathway. EMBO J. **9:**2179–2184.
24. **Münger, K., W. C. Phelps, V. Bubb, P. M. Howley, and R. Schlegel.** 1989. The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes. J. Virol. **63:**4417–4421.
25. **Münger, K., B. A. Werness, N. Dyson, W. C. Phelps, E. Harlow, and P. M. Howley.** 1989. Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. EMBO J. **8:**4099–4105.
26. **Münger, K., C. L. Yee, W. C. Phelps, J. A. Pietenpol, H. L. Moses, and P. M. Howley.** 1991. Biochemical and biological differences between E7 oncoproteins of the high- and low-risk human papillomavirus types are determined by amino-terminal sequences. J. Virol. **65:**3943–3948.
27. **Pagano, M., G. Draetta, and P. Jansen-Dürr.** 1992. Association of cdk2 kinase with the transcription factor E2F during S phase. Science **255:**1144–1147.
28. **Phelps, W. C., S. Bagchi, J. A. Barnes, P. Raychaudhuri, V. Kraus, K. Münger, P. M. Howley, and J. R. Nevins.** 1991. Analysis of *trans* activation by human papillomavirus type 16 E7 and adenovirus 12S E1A suggests a common mechanism. J. Virol. **65:**6922–6930.
29. **Phelps, W. C., K. Münger, C. L. Yee, J. A. Barnes, and P. M. Howley.** 1992. Structure-function analysis of the human papillomavirus type 16 E7 oncoprotein. J. Virol. **66:**2418–2427.
30. **Phelps, W. C., C. L. Yee, K. Münger, and P. M. Howley.** 1988. The human papillomavirus type 16 E7 gene encodes transactivation and transformation functions similar to adenovirus E1a. Cell **53:**539–547.
31. **Qin, X.-Q., T. Chittenden, D. M. Livingston, and W. G. Kaelin, Jr.** 1992. Identification of a growth suppression domain within the retinoblastoma gene product. Genes Dev. **6:**953–964.
32. **Rawls, J. A., R. Pusztai, and M. Green.** 1990. Chemical synthesis of human papillomavirus type 16 E7 oncoprotein: autonomous protein domains for induction of cellular DNA synthesis and for *trans* activation. J. Virol. **64:**6121–6129.
33. **Schreiber, M., P. Mathias, M. M. Müller, and W. Schaffner.** 1989. Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells. Nucleic Acids Res. **17:**6419.
34. **Shirodkar, S., M. Ewen, J. A. DeCaprio, J. Morgan, D. M. Livingston, and T. Chittenden.** 1992. The transcription factor E2F interacts with the retinoblastoma product and a p107-cyclin A complex in a cell cycle-regulated manner. Cell **68:**157–168.
35. **Stirdivant, S. M., H. E. Huber, D. R. Patrick, D. Defeo-Jones, E. M. McAvoy, V. M. Garsky, A. Oliff, and D. C. Heimbrook.** 1992. Human papillomavirus type 16 E7 protein inhibits DNA binding by the retinoblastoma gene product. Mol. Cell. Biol. **12:**1905–1914.

36. **Storey, A., K. Osborn, and L. Crawford.** 1990. Co-transformation by human papillomavirus types 6 and 11. J. Gen. Virol. **71:**165–171.
37. **Storey, A., D. Pim, A. Murray, K. Osborn, L. Banks, and L. Crawford.** 1988. Comparison of the in vitro transforming activities of human papillomavirus types. EMBO J. **7:**1815–1820.
38. **Watanabe, S., T. Kanda, H. Sato, A. Furuno, and K. Yoshiike.** 1990. Mutational analysis of human papillomavirus type 16 E7 functions. J. Virol. **64:**207–214.
39. **Watanabe, S., H. Sato, N. Komiyama, T. Kanda, and K. Yoshiike.** 1992. The E7 functions of human papillomaviruses in rat 3Y1 cells. Virology **187:**107–114.
40. **zur Hausen, H., and A. Schneider.** 1987. The role of papillomaviruses in human anogenital cancers, p. 245–263. *In* N. Salzman and P. M. Howley (ed.), The Papovaviridae, vol. 2. Plenum Press, New York.