# MINIREVIEW

# Role of Papillomavirus Oncogenes in Human Cervical Cancer: Transgenic Animal Studies (43720A)

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*Abstract.* **Human papillomaviruses are believed to be etiologic agents for the majority of human cervical carcinoma, a common cancer that is a leading cause of death by cancer among women worldwide. In cervical carcinoma, a subset of papillomaviral genes, namely E6 and E7, are expressed. *In vitro* tissue culture studies indicate that HPV E6 and E7 are oncogenes, and that their oncogenicity is due in part to their capacity to inactivate cellular tumor suppressor genes. The behavior of E6 and E7 *in vitro* and the genetic evidence from analysis of human cancers suggest that the E6 and E7 genes play a significant role in the development of cervical cancer. This hypothesis is now being tested using animal models. In this review, we summarize our current knowledge of the oncogenicity of papillomavirus genes that has been generated through their study in transgenic mice.** [P.S.E.B.M. 1994, Vol 206]

Human papillomaviruses (HPVs) are small DNA viruses that infect various epithelial tissues including the skin epidermis and epidermal lining of the anogenital tract. As a result of infection, papillomaviruses in general cause warts, benign lesions in the skin that result from the hyperproliferation of the epidermis. It has long been known that certain papillomaviruses could also cause cancers, dating back to the discovery in the early 1930s that the cotton tail rabbit papilloma virus is associated with tumor formation in its natural host (1, 2). Recently, a subset of anogenital human papillomaviruses which includes the HPV-16, 18, 31, and 33 genotypes has been found to be associated with greater than 90% of cervical carcinomas (for review, see 3). These are the so-called high-risk HPVs as distinguished from the more prevalent "low-risk" anogenital HPVs which cause benign lesions only. According to the World Health Organization, there are approximately 500,000 cases of cervical carcinoma worldwide, and approximately 45% of patients die from this disease, usually as a result of metastases. The finding that certain HPVs are causally associated with this common cancer has led to the increased study of these small DNA tumor viruses over the past decade. This article summarizes our current understanding of how papillomavirus genes are thought to play a role in the development of human cervical carcinoma. Specific emphasis is placed on describing properties of papillomavirus genes that have been identified through their study in transgenic mice.

## A Role for Specific Papillomaviral Genes in Cervical Carcinoma

Analysis of papillomavirus DNA residing in human cervical carcinoma tumors or derived cell lines

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0037-9727/94/2061–0024$10.50/0

led to the identification of specific viral genes that likely play a role in cancer development. Papillomaviral DNA is commonly found to be integrated into the host chromosome in cervical cancers (4, 5). The integration events appear to be random with respect to the host genome, but specific with respect to the viral genome. While internally disrupted, the viral genomes in cervical carcinomas consistently support expression of a subset of viral genes. These genes, E6 and E7, are tandemly arranged in the viral genome and positioned just upstream of the position of the disruptions resulting from integration. Several E6 and E7 specific viral mRNAs can be detected in cervical carcinomas that are differentially spliced (6). These mRNAs have 3′ ends that are cellular in origin and result from splicing events which utilize a 5′ splice signal just downstream of the E7 ORF and different 3′ splice signals present in the neighboring cellular DNA. That E6 and E7 are the only viral genes consistently expressed in cervical cancer led many investigators to turn their attention to studying the activities of these two papillomavirus genes.

## Functions of High-Risk HPV E6 and E7 Proteins *in Vitro*

Tissue culture studies demonstrated that HPVs associated with cervical carcinoma, and specifically the viral E6 and E7 genes, exhibit immortalizing properties in human foreskin keratinocytes (7–11) and alter keratinocyte differentiation (11–15). The E6 and E7 genes are not sufficient to induce tumorigenicity but are able to cooperate with an activated *ras* oncogene to transform baby rat kidney or human cervical epithelial cells (16–19). Subsequent studies demonstrated that the E6 and E7 gene products belong to a family of oncoproteins that appear to affect cell growth and differentiation at least in part through their interactions with cellular tumor suppressor genes. The E7 protein, like Adenovirus E1A protein (20) and the large tumor antigens (Tag) of Simian virus 40 (SV40) (21) and polyomaviruses (22), associates with the retinoblastoma susceptibility gene product, Rb (23). Among the different HPV genotypes, the E7 proteins encoded by the high-risk HPVs (HPV-16 and HPV-18) are most efficient in binding Rb (24, 25). The interactions of these oncoproteins with Rb are thought to affect Rb's activities including its capacity to bind the cellular E2F transcription factor (26, 27) (Fig. 1). The E6 protein, like SV40 Tag (28, 29) and the Adenovirus 55 kd E1b gene product (30), is capable of binding the p53 protein (31), now understood to be a tumor suppressor gene product (for review, see 32). The binding of E6 to p53 is dependent upon another cellular protein, E6-associated protein (E6-AP) (33, 34), and leads to an increased instability of the p53 protein (35) (Fig. 1). Consistently, E6 has been shown to inhibit p53 protein's transcriptional transactivation activity in tissue culture cells (36). Genetic analyses indicate that E7's capacity to bind Rb is necessary for its oncogenic activity in most though not all tissue culture assays (37–40). Furthermore, whereas the Rb and p53 genes are mutationally inactivated in HPV-negative cervical carcinomas they are wild type in HPV-positive cervical carcinomas (41, 42). This provides indirect evidence for the biological importance of E6 and E7 interactions with p53 and Rb in cancer. In sum, the observations to date indicate that E6 and E7 may be potent *trans*-dominant negative effectors of tumor suppressor protein function and that these activities are likely to play a role in the oncogenicity of these viral proteins found expressed in human cervical carcinoma.

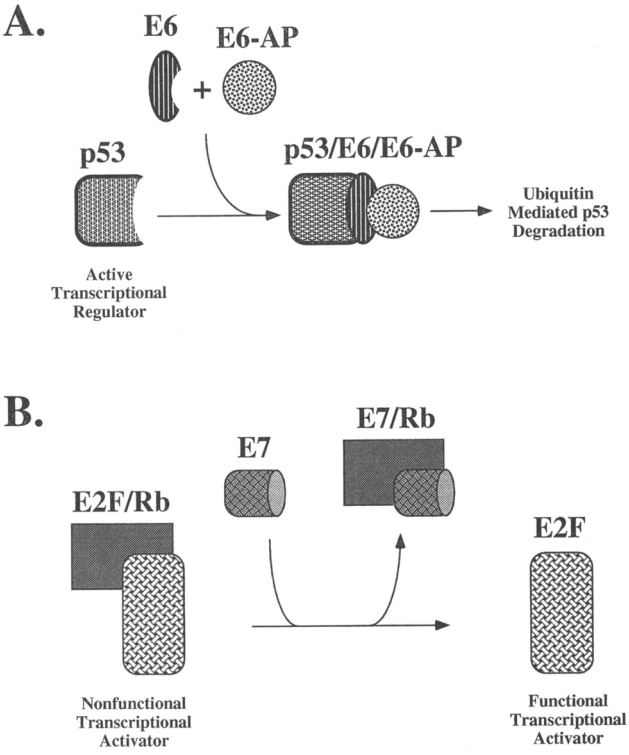


**Figure 1.** Biological consequence of viral oncoprotein interactions with cellular tumor suppressors. (A) Schematic shows mechanism by which E6 is hypothesized to inactivate p53 protein through ubiquitin mediated degradation of p53. (B) Schematic shows role of E7 in altering activity of the cellular transcription factor E2F via its capacity to bind Rb protein.

## Use of Transgenic Animals to Study Oncogene Function *in vivo*

Tissue culture studies have provided important insights into the biological activities of a wide variety of viral and cellular oncogenes; however, in and of themselves, such studies do not provide an accurate picture of an oncogene's role in cancer development. For this reason, investigators have turned to the use of transgenic animals to better assess the contribution of individual oncogenes to carcinogenesis (for reviews see

43, 44). Aberrant expression of cellular proto-oncogenes (45–49) or expression of mutationally activated proto-oncogenes (50) such as those originally isolated from retroviruses results in abnormal cell proliferation and in tumorigenesis (48, 51, 52). Oncoproteins from other DNA tumor viruses, such as SV40 and polyomavirus, also have been shown to be inducers of carcinogenesis in transgenic mice (53–58). In most of these studies, early events in the course of disease often involve the inhibition of cellular differentiation and concomitant induction of abnormal cellular proliferation (56, 57, 59, 60). From these transgenic studies has been confirmed the longstanding hypothesis that cancer evolves from multiple steps involving many genetic changes in cellular genes that control cell growth and differentiation, and that a given oncogene confers part but not all of the changes necessary to induce tumor formation. In this review, we describe the use of transgenic mice for the study of papillomavirus genes and their role in cancer. These studies demonstrate that the human papillomavirus E6 and E7 oncogenes implicated in cervical cancer are indeed capable of potentiating tumor formation in animal models (Table I).

## Transgenic Studies: Early Studies on the Biological Activities of Bovine Papillomavirus Type 1

The first success in generating an *in vivo* model for analyzing papillomaviruses and their oncogenic activities in animals came from the study of bovine papillomavirus Type 1 (BPV-1) in transgenic mice. Over the last half decade a wealth of information has been obtained from the study of these BPV-1 transgenic mice. BPV-1, a papillomavirus belonging to a subgroup that causes fibro-papillomas in their natural hosts, has been the prototype papillomavirus for molecular genetic studies owing to its capacity to transform mouse cells

**Table I.** Summary of Phenotypes of Transgenic Mice Carrying Papillomavirus Genes

| Mouse lineage | Viral genes | Sites of expression | Phenotypes | References |
|---|---|---|---|---|
| BPV1.69 | BPV-1 genome (complete) | Skin | Dermal fibroblast hyperplasia—fibromatosis occurs at sites of wounding, correlates with appearance of extrachromosomal BPV-1 genomes, and expression of viral genes; Fibrosarcomas—correlates with karyotypic changes, altered expression of *jun* proto-oncogenes, and release of bFGF | 62–66 |
| αcry-HPV16E6/E7 Line 4, 18, and 19 | HPV-16 E6 & E7 | Lens | Microphthalmia—inhibition of lens fiber cell differentiation, induction of lens epithelial cell differentiation; Lens tumors—Line 4 and 19 only—locally invasive, poorly differentiated | 68 (also this reference) |
| αcry-HPV16E6/E7 Line 19 | HPV-16 E6 & E7 | Skin | Abnormal skin—characterized by local areas of epidermal hyperplasia; Skin tumors—primarily squamous cell carcinomas, occurring at sites of abnormal skin and/or sites of wounding, correlates with high level of viral gene expression, absence of mutations in Rb and p53 | 80 |
| Actin-HPV16E6/E7 | HPV-16 E6 & E7 | Brain | Neuroepithelial carcinomas—correlated with expression of viral genes, absence of mutations in Rb and p53 | 79 |
| MMTV-HPV16E6/E7 Line 181, 261, and 274 | HPV-16 E6 & E7 | Testes | Seminoma—arising from putative germ line cell lineage and expressing viral genes | 71 |
| K6-HPV1e | HPV-1 early genes | Skin | Abnormal epidermal differentiation—dysplasia and hyperplasia of the suprabasal layers with both hyperkeratosis and focal parakeratosis in the stratum corneum, correlates with expression of E6/E7 and E4 mRNAs | 81 |

in tissue culture via infection or DNA transfection (for review see 61). Dr. D. Hanahan generated mice in which multiple copies of the intact BPV-1 genome were introduced into the germ line of mice through microinjection of DNA into the pronuclei of fertilized mouse eggs. The viral DNA was integrated into the mouse genomic DNA and stably inherited in all cells of the resulting animal. One line of mice developed fibrosarcomas (62) which occurred late in adult life. Earlier preneoplastic lesions often arising at sites of wounding could be detected in these animals; viral genes were found to be expressed in these preneoplastic lesions as well as in the neoplasias.

One of the most surprising properties of the BPV-1 DNA present in these transgenic mice was its capacity to replicate extrachromosomally in the preneoplastic and neoplastic lesions of the skin (62, 63). This plasmid-like state of the viral genome presumably resulted from a recombination event in which an integrated copy of viral DNA present in the mouse genome, perhaps during its own replication, recombined to form a circular daughter species. The recombination event may have been facilitated by the fact that the Hanahan laboratory originally generated the transgenic mice by microinjecting a DNA fragment containing 1.69 copies of the viral genome, thereby providing a partial direct repeat (62). The maintenance of high copies of the viral plasmid in these mouse cells, not only indicates the need for expression of the viral E1 and E2 replication proteins, but also suggests that viral genomic amplification may be an important factor in the development of the preneoplastic lesions. Indeed, the level of viral gene expression was heightened only in those tissues in which viral DNA amplification could be found (63).

It is not clear which viral genes contribute to the oncogenic process observed in these BPV-1 transgenic mice. It is presumed, based upon prior tissue culture studies, that either or both the E5 and E6 oncogenes of BPV-1 play a primary role. These two BPV-1 genes have been demonstrated in tissue culture to be independently acting transforming genes (61); and both genes were found to be expressed in the fibrosarcomas that arose in the BPV-1 transgenic mice (63). The levels of viral gene expression in the preneoplastic fibromatoses and neoplastic fibrosarcomas in these BPV-1 transgenic mice was equivently induced compared with unaffected transgenic skin (63). This led to the hypothesis that additional genetic events, presumably affecting cellular genes, must be required for the development of neoplasms. Cytogenetic analyses led to the identification of several recurring genetic changes in the mouse chromosomes in the cells from the fibrosarcomas (64), specifically loss of Chromosome 14 and duplications of regions in Chromosome 8. Recently, it has been demonstrated that increased levels of expression of cellular nuclear oncogenes, *jun*B and c-*jun*, occur in the aggressive fibromatoses and in fibrosarcomas in these BPV-1 transgenic mice (65). In addition the secretion of bFGF in the fibromasarcomas has been argued to play a role in the angiogenic properties of these tumors (66). In sum, the BPV-1 transgenic mice provide one of the more thoroughly studied models for virally induced carcinogenesis to date.

## Transgenic Studies: Evaluating *in Vivo* Properties of High-Risk HPV Oncogenes

The success in studying the biological activities of BPV-1 *in vivo* led many scientists to attempt similar studies with HPV genomes. Early studies, like the original BPV-1 experiments, involved the introduction of the full-length HPV genomic DNAs, or tandem copies thereof, into the mouse germ line. Despite numerous attempts by several laboratories, this approach by and far led to unsatisfying results. Of those full-length genomic HPV-16 transgenic mice generated, none had highly penetrant, overt phenotypes. This disparity between the biological activities of the intact BPV-1 and the HPV genomes in mice was somewhat predictable. Whereas HPVs have little effect on normal rodent cells in tissue culture upon transfection of viral DNA, BPV-1 had long been known to have strong effects both in transforming infected or transfected rodent cells in tissue culture and in causing tumors in infected rodent animals (for review, see 61). Furthermore, BPV-1 can replicate extrachromosomally in mouse cells (67). Presumably, these differences are caused, at least in part, by differences in the transcriptional activity of the viral promoters in rodent cells, since direction of HPV E6 and E7 gene expression from strong heterologous promoters can lead to detectable expression of the HPV genes in and altered growth properties of rodent cells.

The use of heterologous transcriptional promoters has now led to successes in studying HPV genes in transgenic mice. As summarized in the introduction to this review, the early analyses of cervical cancers had led to the understanding that the E6 and E7 genes of the high-risk HPVs are likely to be important etiological agents in the development of this common cancer. Several investigators have now succeeded in evaluating the biological activities of the E6 and E7 genes in transgenic mice. The results of these studies are discussed below.

## E6 and E7 Expression in the Mouse Ocular Lens

Through the use of the strong tissue specific promoter for the murine αA crystallin gene, the activities of the HPV-16 E6 and E7 genes have been studied in mouse ocular lens (68). This transcriptional promoter provides the important property of directing transgene expression in the developing lens tissue during embryogenesis, as well as throughout the adult life of the

animal. Thus the effects of the viral oncogenes on differentiative properties of this simple epithelial tissue can be observed as well as the effects of long-term expression of the oncogenes in adult tissue. Three germline transgenic mice, αAHPV16E6/E7 lineages, were generated and evaluated. Expression of E6 and E7 in the lens resulted in the efficient inhibition of normal lens cell differentiation, and the coincident induction of lens epithelial cell proliferation (68) (Fig. 2). These properties were evident as early as day 16 in embryogenesis, and persisted into the adult life of the animal. Grossly, these adult animals displayed microphthalmia and cataracts with 100% penetrance in all three lines of mice. Adult mice in the line expressing highest levels of E6 and E7 genes developed eye tumors which were lenticular in origin (68) (Fig. 3). Similar tumors have been found to occur less frequently in another αAHPV16E6/E7 lineage, Line 4 (Fig. 3). These tumors were poorly differentiated, locally invasive, highly vascularized, and expressed the viral E6 and E7 oncogenes (68) (Fig. 3). In addition, the preneoplastic lens cells from these transgenic mice, when placed in tissue culture, displayed an immortalized phenotype, and upon continued passage, acquired tumorigenic characteristics (68). The long latency of tumor incidence both in these mice and in derivative cell lines again supports the hypothesis that additional genetic or epigenetic changes are required for tumorigenesis.

The early phenotype seen in the lens of αAHPV16E6/E7 mice bears certain similarities to the biological activities of E6 and E7 when expressed in tissue culture. As occurs in the lens, E6 and E7 have been shown to inhibit the differentiation of human foreskin keratinocytes. This has been best demonstrated in organotypic culture (11). Also, the induction of cellular proliferation seen in the lens (68) may correlate with the immortalization phenotype seen in tissue culture for human epithelial cells transfected with high risk HPV E6 and E7 (9, 10). Importantly, neither in the animals nor in tissue culture are E6 and E7 found to be sufficient for neoplastic development, though in each case, they clearly potentiate the later acquisition of the transformed or tumorigenic phenotype.

## E6 and E7 Expression in the Skin

Ectopic expression of the E6 and E7 transgenes in the skin of the αAHPV16E6/E7 transgenic mice has provided a second tissue in which to study the activities of these viral oncogenes. Expression of E6 and E7 was found to correlate with a high incidence of hyperplastic epidermal skin lesions and the subsequent development of Grade 1–3 epidermoid carcinomas (80). An interesting property of these skin cancers is that at least some arise at sites of wounding (80) (Fig. 4), much like what occurred in the BPV-1 transgenic mice. Indeed, wounding, as well as other events that may lead to induction of epithelial cell proliferation, has been argued to play a role in many cancers. For instance, in *ras* transgenic mice, sites of wounding were found to be susceptible to the formation of papillomas (82).

The parallels between the skin disease seen in the αAHPV16E6/E7 transgenic mice, and the multistage development of cervical carcinoma in patients infected with anogenital papillomaviruses strongly argue that E6 and E7 can be powerful etiological agents in the development of squamous cell carcinoma. Specifically, one finds expression of the viral transgenes in these transgenic mice to be low in the preneoplasias and high in the squamous cell carcinomas. An increase in the expression of E6 and E7 has been hypothesized to play an important role in the incidence of cervical cancer. *In situ* hybridization analyses of viral gene ex-

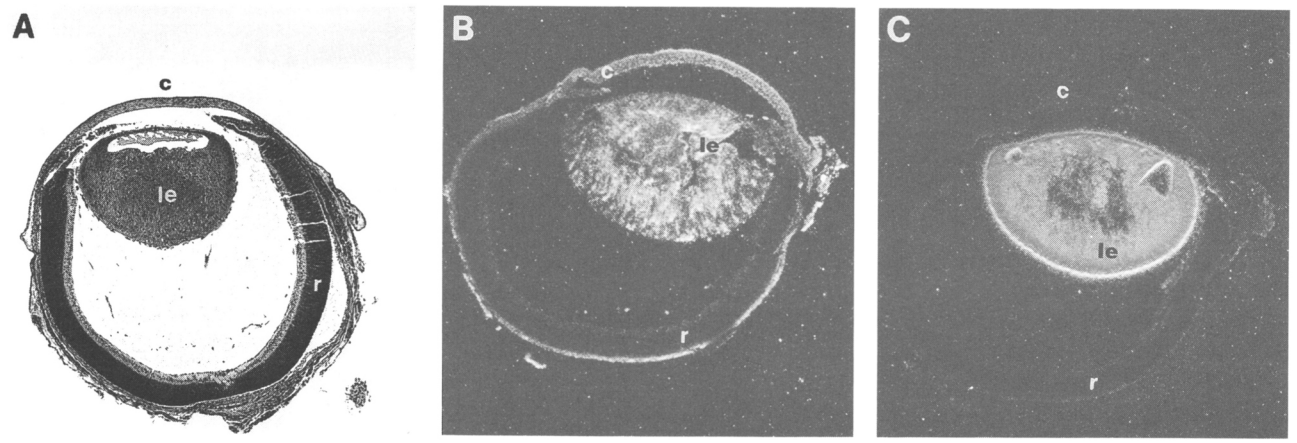


**Figure 2.** Expression of E6/E7 in the lens of αAHPV16E6/E7 Line 19 transgenic mice. Paraffin embedded eyes from a neonatal Line 19 mouse were sectioned at 5 μ thickness. (A). Histological section stained with hematoxylin-eosin. (B). *In situ* hybridization of a section to a $^{35}$S-labeled antisense E6/E7 specific cRNA probe. Shown is the E6/E7 expession pattern in dark field illumination. (C). *In situ* hybridization of a neighboring section to a $^{35}$S-labeled antisense specific α crystallin cRNA probe. Shown is the α crystallin expression in dark field illumination. Note the overlap with E6/E7 expression specifically in the lens. *In situ* hybridization was performed as in Ref. 80. Magnification ×5. c, cornea; le, lens; r, retina.

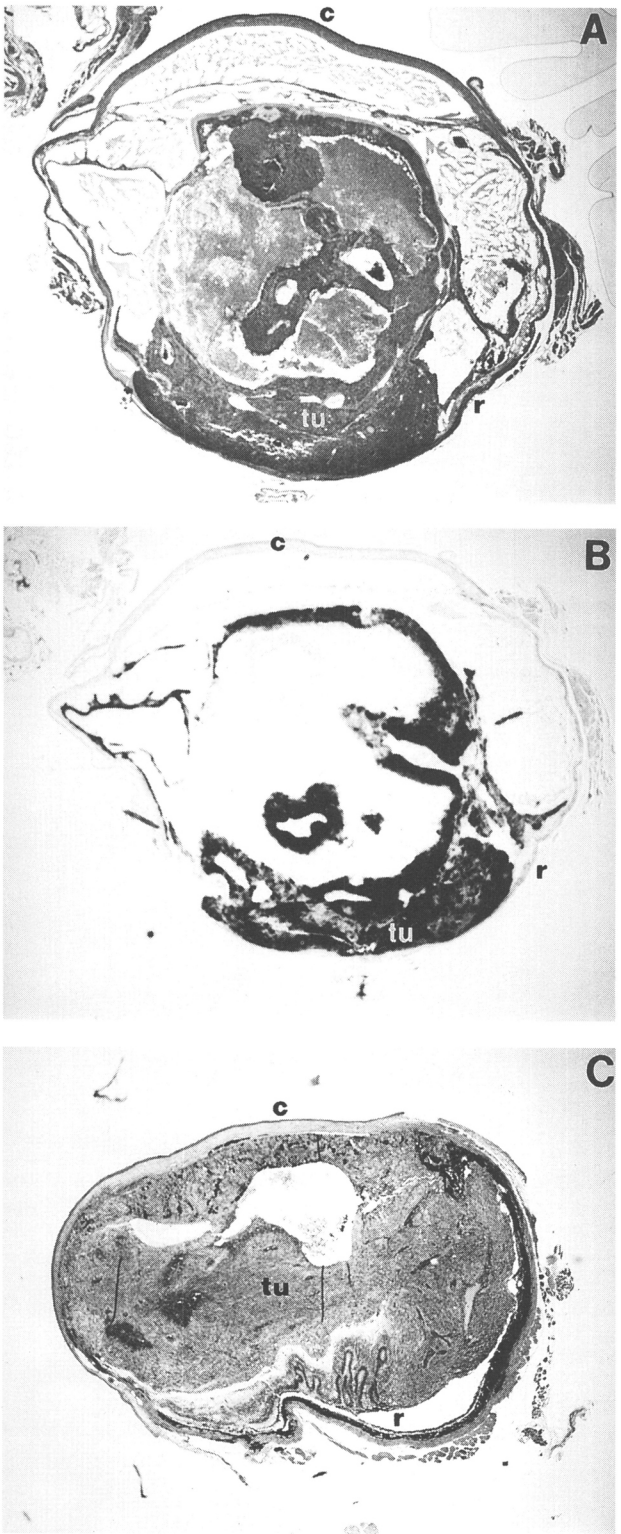


**Figure 3.** Lens tumor in adult αAHPV-16E6/E7 mice. Paraffin embedded eyes were sectioned at 5-μ thickness. (A). Histological section of a Line 19 eye containing a lens tumor stained with hematoxylin-eosin. (B). *In situ* hybridization of a neighboring section of the eye tumor in (A) to a $^{35}$S-labeled antisense specific α crystallin cRNA probe. Shown is the α crystallin expression in bright field illumination. Note expression of α crystallin in the tumor. (C). Histological section of a Line 4 eye containing a lens tumor stained with hematoxylin-eosin. Note detached retina. *In situ* hybridization was performed as in Ref. 80. Magnification ×2.5. c, cornea; r, retina; tu, lens tumor.

pression in cervical carcinoma lesions suggest that E6 and E7 gene expression is higher in the poorly differentiated epithelial cell types within the carcinoma than in that of preneoplastic lesions (69). High level expression of the viral oncogenes in the skin cancers in these transgenic mice also occurs selectively in the basal or poorly differentiated epithelial cells (80) (Fig. 4). Secondly, this high level transgene expression correlates with positive staining for proliferating cell nuclear antigen (PCNA), a marker for proliferating cells (80). These observations are consistent with that seen in cervical cancers (70) and argue that E6 and E7 induce cell proliferation. Lastly, the Rb and p53 genes were not mutated in these mouse skin cancers (80), as is the case in HPV positive cervical carcinomas (41, 42). This is consistent with the prediction that E6 and E7 activities obviate the requirement for mutational inactivation of these tumor suppressor genes. It has also been recently noted that, as is seen in cervical carcinoma patients, αAHPV16E6/E7 transgenic mice afflicted with squamous cell carcinomas generate a highly penetrant immunological response to endogenously expressed E7 protein (Frazer, Leippe, Tindle, Liem, Fernando, Phelps, and Lambert, manuscript in preparation). In sum, these many parallels indicate that these mice may be an accurate model for studying the role of E6 and E7 in human cancer.

The induction of epidermal hyperplasia in the skin by HPV-16 E6 and E7 seen in the αAHPV16E6/E7 transgenic mice has now been reproduced in transgenic mice carrying the HPV-16 oncogenes expressed from the human keratin 14 gene promoter (Herber and Lambert, unpublished observations). Relatedly, expression of HPV-1 early genes from a bovine keratin promoter led to alterations in epidermal differentiation (81). This correlated with the expression of E6 and E7 as well as E4 containing mRNAs; however, it is not clear which of these genes is necessary and/or sufficient to induce the observed effects by HPV-1.

### E6 and E7 Expression in Genital Tissues

Several studies have demonstrated oncogenic properties of the HPV-16 E6 and E7 genes when expressed in genital tissues of the mouse. Kondoh *et al.* (71) found testicular tumors to arise with high frequency in transgenic mice carrying both E6 and E7 genes of HPV-16 positioned downstream of the mouse mammary tumor virus (MMTV) long terminal repeat (LTR). These testicular tumors, a type of seminoma putatively arising from germ cell lineage, arose in 8–10-month-old mice from three independent germ lines with high penetrance, ranging from 17% (one of six mice) to 100% (five of five mice) of males. As with the expression in the eye and skin of mice, there was observed a long latency period before tumor onset. Expression of the viral genes was seen not only in tu-

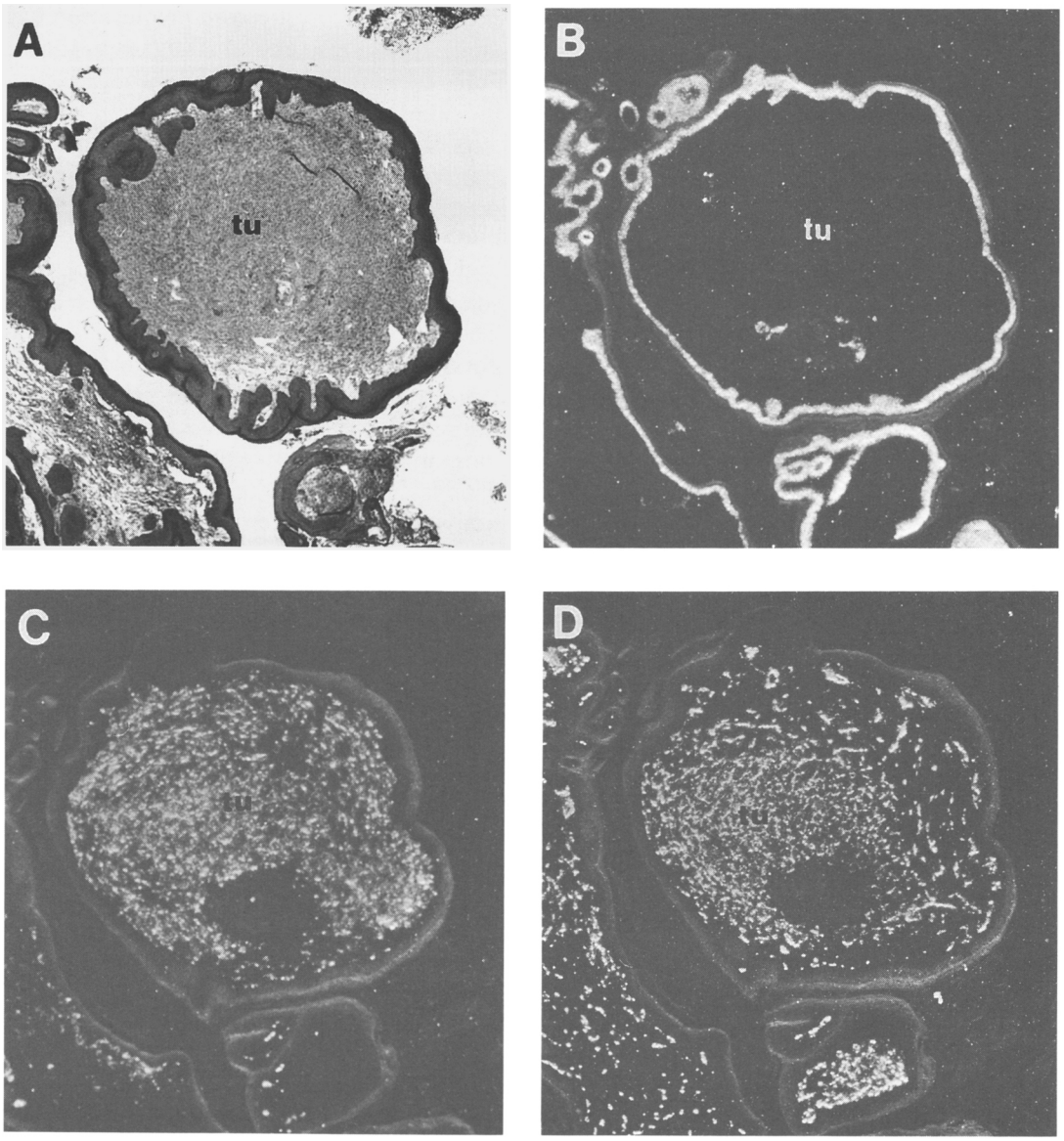


**Figure 4.** Squamous cell carcinoma arising on male genitalia of a line 19 αAHPV-16E6/E7 mouse. This tumor arose at a site of wounding associated with fighting between male littermates. Paraffin embedded tumor was sectioned at 5-μ thickness. (A) Histological section of a portion of the tumor stained with hematoxylin-eosin. (B) *In situ* hybridization of a neighboring section of the tumor in (A) to a $^{35}$S-labeled antisense specific Type 1 transglutaminase cRNA probe. Shown is the Type 1 transglutaminase expression in dark field illumination. Note expression of Type 1 transglutaminase in the differentiated portion of the epidermis surrounding the tumor. (C) *In situ* hybridization of a neighboring section of the tumor in (A) to a $^{35}$S-labeled antisense specific E6/E7 cRNA probe. Shown is the E6/E7 expression in dark field illumination. Note expression of E6/E7 throughout the tumor. (D) *In situ* hybridization of a neighboring section of the tumor in (A) to a $^{35}$S-labeled antisense specific α1 Type IV collagen cRNA probe. Shown is the α1 type IV collagen expression in dark field illumination. Expression of α1 type IV collagen, a marker for undifferentiated epidermal cells, is seen throughout the tumor. Note the similarity in the pattern of expression of E6/E7 and α1 Type IV collagen genes, indicating that E6/E7 are expressed in the poorly differentiated epidermal cells of the tumor. *In situ* hybridization was performed as in Ref. 80. Magnification ×2.5. tu, squamous cell carcinoma.

mors, but in tissues that did not exhibit histological abnormalities. This observation may indicate that E6 and E7 gene products display cell-type specificity in their capacity to potentiate tumorigenesis. Relatedly, these lines of mice did not develop mammary tumors in females as one might predict based upon the tissue specificity of the MMTV. Mammary gland tumors have been demonstrated to arise in transgenic mice carrying the *neu* (72, 73), *int*-1 (74), *int*-2 (75), c-*myc* (45) and *H-ras* (76) oncogenes under the transcriptional control of the MMTV LTR. However, the MMTV LTR appears to lack tissue specificity when used to direct expression of transgenes in mice based upon previous transgenic studies (77). It is not clear whether the paucity of mammary tumors in the MMTV/HPV16 E6E7 transgenic mice correlates with the presence or absence of expression of the transgenes in this tissue.

An alternative approach for directing expression of the papillomavirus oncogenes to the genital tissue of the mouse has been recently described which employed directed infection of the mouse cervix with recombinant retroviruses (78). While not a germline transgenic experiment, this approach provided useful information into the biological properties of HPV-16 E6 and E7 genes in the appropriate tissue type for this anogenital papillomavirus. In this study, the HPV-16 E6 and E7 genes along with the upstream HPV-16 noncoding region (NCR) was inserted into a retroviral vector. Infection of mouse cervix led to a large number of highly dysplatic lesions, in which there was found presence and expression of viral genes. In this animal model, frank cancers did not develop unless the animals' infected tissue was also treated with chemical carcinogens; this led to a synergistic induction of squamous cell carcinomas. The HPV-16 NCR contains the transcriptional control signals required for the expression of E6 and E7 from the intact papillomavirus genome. It is not clear, however, why in these retroviral infection experiments there was found expression of the viral oncogenes in the cervix, while in the earlier transgenic studies in which the intact HPV-16 genome was introduced into animals, there was neither expression nor pathological phenotypes associated with the presence of the HPV genome in the mouse cervix. One possibility is that the retroviral LTR, positioned upstream of the papillomaviral DNA insert within the retroviral vector, contributed to the expression of the E6 and E7 genes.

### E6 and E7 Expression in the Brain

The use of the ubiquitously active β-actin transcriptional promoter to direct expression of the HPV-16 E6 and E7 genes led to the frequent observance of brain tumors in one line of transgenic mice (79). These tumors arose in adult mice and expressed viral E6 and E7 RNAs, and E7 protein. The tumor types detected in this line of mice included anaplastic neuroepithelial tumors, well differentiated choroid plexus carcinomas, and rare pituitary carcinomas. Interestingly, other papovaviruses, specifically SV40 (53) and lymphotropic papovavirus (60), have previously been found to cause choriod plexus tumors. As is true for the other transgenic studies summarized above, it is unclear what factors are influencing the tissue specificity for tumorigenesis in this line of mice beyond the particular tissue pattern of transgene expression. The tumors were found to contain normal levels of p53 and phosphorylated Rb protein. These results are consistent with the absence of inactivating mutations in these cellular tumor suppressor genes.

### Summary

From the transgenic studies summarized, several conclusions can be drawn concerning the biological activities of high-risk HPV E6 and E7 oncogenes: (i) these oncogenes are capable of potentiating tumorigenesis in a number of epithelial cell types in mice; (ii) the primary activity of E6 and/or E7 on epithelial cells is the inhibition of differentiation and induction of proliferation; (iii) the latency of tumor formation in these mice argues for the need for additional genetic events in tumorigenesis; and (iv) the absence of mutations in p53 and Rb in the tumors arising in these animals argues for the role of E6 and E7 in functionally inactivating these cellular tumor suppressor gene products.

### Future Directions

An important next step in the study of HPV oncogenes is to identify the individual biological activities of the E6 and E7 genes *in vivo*. Due to the highly penetrant and reproducible phenotypes seen in transgenic studies using the αA crystallin promoter, lens directed expression of the individual viral oncogenes should provide useful information in this regard. Such transgenic mice have now been evaluated. Findings indicate that HPV-16 E7 protein induces cell proliferation, programmed cell death, and inhibits lens fiber cell differentiation (Pan and Griep, manuscript submitted), activities that correlated with the capacity of E7 protein to associate with the Rb protein. In contrast, expression of HPV-16 E6 inhibited lens fiber cell denucleation but failed to induce cell proliferation. Additional studies now in progress will determine whether the individual activities of E7 in the lens is due solely to its capacity to inactivate Rb, and whether E6 or E7 alone, versus together, can lead to tumorigenesis. It is important to remember, in this context, that the capacity of E7 to bind and inactivate Rb protein does not always correlate with its transforming activity in tissue culture (76), raising the possibility that E7 possesses other activities that are necessary, if not sufficient for transformation. Secondly, it will also be important to compare and contrast the *in vivo* activities of the E6 and E7 genes from high-risk versus low-risk anogenital papillomaviruses in an effort to identify the properties that determine oncogenic potential.

The skin remains the tissue of choice for studying papillomavirus genes, owing to the tissue tropism of these viruses for the skin in their natural hosts, and the recent availability of epithelial specific keratin promoters should permit more detailed genetic dissection of the role of E6 and E7 in skin carcinogenesis. As indicated above, recent studies indicate the likely success in genetically dissecting E6 and E7 functions required for alteration of epidermal cell growth and differentiation, and the induction of squamous cell carcinomas in the skin of transgenic mice.

The transgenic studies summarized above establish E6 and E7 as causitive agents of epidermal neoplasia. An important direction now being taken is the

identification of cellular genes that are targets for mutational alteration during papillomavirus associated tumorigenesis. Transgenic mice should be invaluable reagents in addressing this goal. The advent of new technologies, including the use of Simple Sequence Length Polymorphisms and Single Strand Conformational Polymorphisms, should help make these analyses more rapid. HPV transgenic mice may also permit the investigation of virus-host interactions pertinent to tumorigenesis. Specifically, E6 and E7 have been implicated as immunological targets for host response in HPV infected patients, and as potential targets for immunological therapy in cervical cancers. The study of HPV-16 E6 and E7 transgenic mice, and syngeneic animals challenged with tumors derived from these transgenic mice may provide new insights into the role of E6 and E7 as targets of the immune system.


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