American Journal of Pathology, Vol. 142, No. 4, April 1993

Neuroepithelial Carcinomas in Mice Transgenic with Human Papillomavirus Type 16 E6/E7 ORFs

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The effect of the human papillomaviral (HPV) oncogenes E6 and E7 was examined in transgenic mice with a construct containing the human β-actin promoter regulating HPV16 E6 and E7 open reading frames. In the sole line of mice that transmitted the transgene, neuroepithelial tumors appeared at 2.5 months of life, and by 10 months, 87 of 122 (71%) of the animals were dead from brain tumors. The most frequent type of tumor (74%) was an anaplastic neuroepithelial tumor associated with the ependyma of the third and fourth ventricles, which locally invaded adjacent brain tissue and spread for considerable distances along the ventricular surface. The other two types of tumors were well-differentiated choroid plexus carcinomas (26%) and rare pituitary carcinomas (8.7%). HPV16 E6 RNA and E7 oncoprotein expression were demonstrated in tumor tissue and primary cell lines derived from the tumors. Examination of two tumor suppressor gene products, the retinoblastoma protein and p53, known to bind to HPV16 E7 and E6 oncoproteins, respectively, showed both were expressed in the primary tumor cell lines. These data support a causative role for the HPV oncoproteins in epithelial carcinogenesis. (Am J Pathol 1993, 142:1187–1197)

Human papillomaviruses (HPVs) cause benign proliferative epithelial lesions. A subgroup of these viruses is associated with anogenital lesions; HPV types 6 and 11 are found in condylomata, while types 16, 18, 31, 33, and 35 are found both in benign lesions and in 90% of uterine cervical and anal cancers.[1] In premalignant lesions viral DNA is a multicopy episome, while in cancers a variable fraction of the viral DNA is integrated into cellular chromosomes.[2,3] Integration disrupts the open reading frames (ORFs) of the viral repressors E1 and E2 but leaves the 5′ E6 and E7 genes intact.[2–4] In HPV-associated cancers there is invariable E6 and E7 expression.[5]

The E6 and E7 genes of cancer-associated HPVs encode two oncoproteins, the transforming properties of which have been revealed by a number of different assays. The E7 gene by itself can transform established rodent fibroblasts and in primary cells can cooperate with an activated *ras* oncogene for transformation.[6] The E6 ORF together with E7 is sufficient for the immortalization of primary human keratinocytes.[7–9] These oncoproteins have been shown to bind and interact with two cellular tumor suppressor gene products, pRB and p53.[10] E7 can bind to the unphosphorylated form of pRB, presumably sequestering it and preventing its activity during G_1/S (10–12). The E6 protein can bind to p53 and facilitate its degradation by the ubiquitin pathway.[10, 13, 14]

To date there has been little published work investigating tumorigenesis in mice transgenic with HPV oncogenes. A recent report documented the occurrence of seminomas in animals transgenic with HPV16 E6/E7 DNA regulated by the mouse mammary tumor virus promoter.[15] In this report we describe a line of mice which are transgenic with a human β-actin–regulated HPV16 E6/E7 construct. The goal was to achieve diffuse expression of the E6/E7 oncoproteins in multiple lines of mice. One line of mice was obtained, and 71% of these animals developed three types of neuroepithelial tumors between 4 and 10 months of age. Further detailed pathologic examination revealed clusters of abnormal neuroepithelium at 1 month of age and microscopic anaplastic

Supported by grant PRTF-121 from the American Cancer Society to J.M.A., grant CA47632 from the National Cancer Institute, and funds from the Markey Charitable Trust for supplies and equipment to D.H.

Accepted for publication September 28, 1992.

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tumors in 20% of the brains studied 2.5 months after delivery. Expression of both HPV16 E6 RNA and E7 oncoprotein, restricted to abnormal tissues, was demonstrated in the tumors and brains of effected animals.

Materials and Methods

Transgenic Mice

The construct used in the study, p1321, has been described previously.[9] The plasmid contains the HPV16 E6/E7 ORFs from nucleotides 79 to 884 inserted into the *Sal*I/*Hind*III site of p1318, a vector which contains 2 kb of 5′ human β-actin enhancer/promoter and 1.5 kb of 3′ human β-actin sequence including the polyadenylation signal.[9,16] The construct was linearized with *Aat*II and microinjected into B6D2/F2 embryos.[17] The line was maintained as heterozygotes by backcrossing to C57Bl6/J animals.

Pathology

Mice were perfused with 4% paraformaldehyde in calcium/magnesium-free phosphate-buffered saline via the left ventricle. The cranium was excised, partially opened, and postfixed for 2 days at 4 C. The brains were excised, coronally sectioned, embedded in paraffin, and stained with hematoxylin and eosin.

Southern Blotting

Tumor and normal tissues were snap frozen in liquid nitrogen and pulverized, the powder was digested overnight with proteinase K, and high-molecular-weight DNA was isolated by standard techniques.[18] The DNA was digested with several restriction enzymes: *Hind*III, which cuts once within the transgene; *Msp*I and *Hpa*II, which are isoschizomers differentially sensitive to methylation, each of which potentially cuts the transgene at multiple sites; and *Sal*I and *Eco*RI, which both cut the transgene twice. Five micrograms of digested DNA were run on 0.8% agarose gels; Southern transfer and transgene detection, using a 750-bp fragment encompassing the full length of the HPV16 E6/E7 ORFs, were performed using standard techniques.[18]

RNA PCR

Samples were taken from multiple organs and snap frozen in liquid nitrogen. The tissue was pulverized, and RNA was isolated using the technique of Chomczynski and Sacchi.[19] One microgram of total RNA was first treated with 10 U of DNase, phenol extracted, ethanol precipitated, and then reversed transcribed using Moloney murine leukemia virus reverse transcriptase. The 20-μl reaction was diluted to 100 μl with water, and the complementary DNA (cDNA) from 5 μl of the diluted reaction was amplified for 40 cycles with primers specific for HPV16 E6 and β_2-microglobulin RNA. The sense HPV16 E6 primer AGAACTGCAATGTTTCAGGACCCACAG spanned nucleotides 95 to 121; the antisense E6 primer TCTGCAACAAGACATACATCGACCGG was from nucleotide 501 to nucleotide 526. The E6 splice donor and splice acceptor sites at nucleotides 226 and 409 would be included in this region, so either full-length E6 of 431 bp or E6* of 245 bp could be amplified.[5,20] The β_2-microglobulin primers were: sense, GCTATCCAGAAGAAACCCCTCAAATTC; antisense, CATGTCTCGATCCCAGTAGACGGTC. They spanned exons I–II and II–III, respectively, and would be expected to amplify a 302-bp cDNA fragment. Aliquots of the cDNA polymerase chain reaction (PCR) were run on 5% NuSieve 3:1 agarose gels, and the fragments were visualized by ethidium bromide staining. The identities of the E6 fragments were confirmed by Southern blotting.

Protein Expression

To detect E7 protein in tissues, samples of tumor and normal tissue were snap frozen in liquid nitrogen, pulverized, and extracted in a lysis buffer containing 1% Nonidet P-40, 100 mM Tris (pH 8.0), 100 mM NaCl, 2 mM EDTA, 1 mM dithiothreitrol, with 0.1 mg/ml phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin/leupeptin, 5 mM sodium fluoride, and 0.1 mM sodium orthovanadate added as proteinase and phosphatase inhibitors. The samples were precleared by centrifugation at 15,000 × *g* and cleared using normal rabbit serum followed by protein A–sepharose collection.

The samples were immunoprecipitated with either a polyclonal HPV16 E7 antibody (a gift of Dr. Denise Galloway, Seattle, WA) or normal rabbit serum overnight at 4°C, the immunoprecipitates were collected on protein A–sepharose, and the washed beads were boiled and the proteins were separated on 14% sodium dodecyl sulfate (SDS)-polyacrylamide gels. The proteins were electrophorectically transferred to polyvinyldifluoride membranes (Immobilon, Millipore, Bedford, MA), and the 18-kd E7 protein

was detected using a monoclonal antibody to HPV16 E7 (Triton Bioscience, Alameda, CA) and visualized with the ECL system (Amersham, Arlington Heights, IL).

To detect pRB and p53, cell monolayers were washed in cold phosphate-buffered saline and lysed with a buffer containing 0.1% Nonidet P-40, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.0), and 250 mM NaCl, with the same collection of proteinase and phosphatase inhibitors described above. Protein concentration was estimated (BioRad, Richmond, CA), and 100 μg of total protein were electrophoresed on 7.5% and 12% SDS-polyacrylamide gels for pRB and p53 detection, respectively. After electrophoretic transfer to polyvinyldifluoride membranes, pRB and p53 were detected with the appropriate monoclonal antibodies (pRB: G3-245 PharMingen, San Diego, CA; p53: Ab-3, Oncogene Science, Uniondale, NY) followed by the ECL system.

Primary Cell Lines

Either anaplastic or ventricular tumors were dissected from surrounding normal brain tissue, minced, and digested with a solution of collagenase D (1 mg/ml) in L10 medium with 10% fetal calf serum. The cells were grown in Dulbecco's modified minimal essential medium with 10% fetal calf serum, penicillin (50 mg/ml), and streptomycin (50 μg/ml). When used in the experiments the cells had been in culture for 6 to 8 months and were passaged 15 to 20 times.

Results

Transgenic Mice

Two transgenic mice were obtained following DNA microinjection of fertilized eggs. One of these was mosaic and never transmitted the transgene. This animal died while giving birth at 1 year of age and otherwise appeared normal. An autopsy was not performed because the death was not discovered for 48 hours. The other mouse segregated the transgene in Mendelian fashion, and the line was maintained as heterozygotes in a C57Bl6/J background. This founder animal died at 6 months of age without autopsy. From March 2, 1990, to June 28, 1991, there were 122 transgenic offspring produced. This cohort was followed until December 31, 1991, and during this interval of 1.5 years 87 (71%) animals died at 4 to 10 months of age. At the time of death all of these animals had developed bulging skulls, lethargy, and abnormal movements. The affected animals died within a week of the appearance of these signs. The most frequent time of occurrence of the tumors was at 5 months.

Pathology

Autopsies were performed on 46 of the 87 animals that died with the above abnormalities. The pathology was found to be restricted to the brain, with all other organs and tissues grossly and microscopically normal. Gross examination of the brains from affected mice showed three different tumors, the frequencies of which are displayed in Table 1. The most common tumor (74%) was in the midline, presenting on the cortical surface between the cerebral hemispheres in the region of the superior and inferior colliculi. The lesions were frequently adherent to the overlying skull. On coronal section these tumors extended from the midbrain to the cortical surface, destroying the overlying tissue. Microscopically, the tumors arose from an area juxtaposed to the choroid plexus, spread diffusely along the surface of the ventricles, and grew out into the adjacent brain parenchyma. The tumors were primitive and anaplastic, with a markedly increased nuclear/cytoplasmic ratio; massively enlarged, bizarre nuclei; and frequent mitotic figures (Figure 1A). There appeared to be an attempt at gland formation in these tumors, and electron microscopy (data not shown) confirmed the presence of tight junctions and microvilli, consistent with an epithelial origin.

The second tumor type appeared as a grapelike mass entirely within the ventricles. These less common tumors (26%) (Table 1) could be found in any of the ventricular cavities but were restricted to one ventricle per individual mouse. Microscopically these tumors were choroid plexus carcinomas, retaining the basic form and shape of the choroid plexus but with frequent mitotic figures and rare local invasion (Figure 1B). Rarely both anaplastic epithelial and choroid plexus carcinomas arose in the

Table 1. *Types and Frequencies of Neuroepithelial Carcinomas Found in Autopsied hAcHPV16E6/E7 Transgenic Mice*

Tumor type	No. (%) with lesion
Anaplastic neuroepithelial carcinoma	34 (74)
Choroid plexus carcinoma	12 (26)
Pituitary carcinoma	4 (8.7)
Total	46

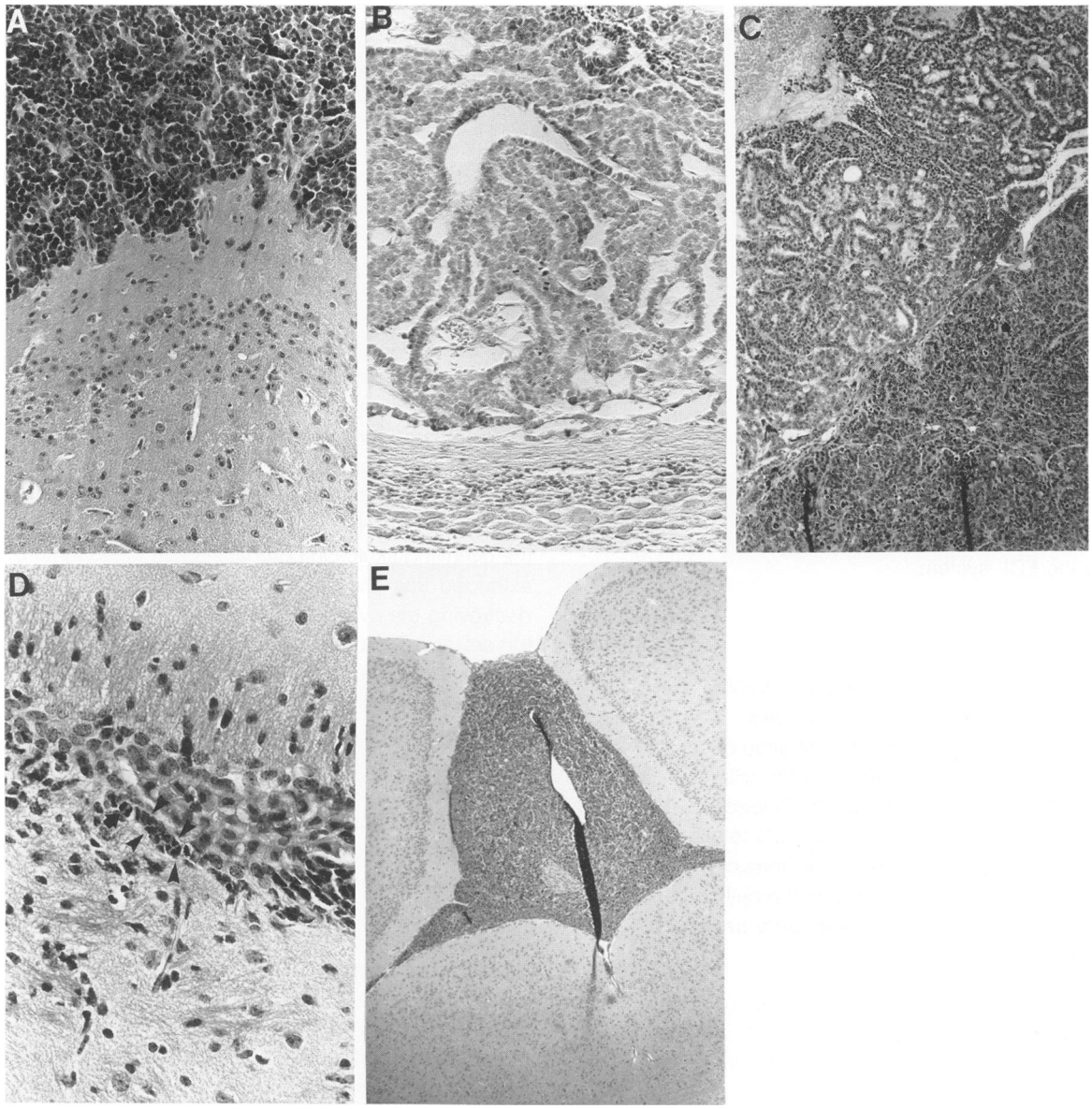

Figure 1. *The histologic appearance of the two most common tumors in β-actin HPV16 E6/7 transgenic mice.* **A**, *H&E-stained anaplastic tumor. Note the bizarre, enlarged nuclei, the frequent mitotic figures, and local invasion into adjacent normal brain (×150).* **B**, *a choroid plexus carcinoma which contains mitotic figures (H&E, ×150).* **C** *is derived from a tumor which appeared in one animal (H&E, ×70). There are both an anaplastic and choroid plexus carcinomas with no transition zone between the two lesions.* **D**, *a hyperplastic focus of anaplastic neuroepithelial cells (outlined in arrowheads) from a 1-month-old transgenic animal. The cells are subependymal and adjacent to normal choroid plexus epithelium. Of note is the bizarre mitotic figure (arrow) present within the focus (H&E, ×300).* **E**, *a microscopic anaplastic tumor in a 2.5-month-old transgenic mouse (H&E, ×60). The animal was asymptomatic.*

same animal (Figure 1C). The third, very infrequent (8.7%) tumor type was an anaplastic pituitary carcinoma (Table 1).

Younger asymptomatic mice were sacrificed at 1, 2.5, and 4 months of age in order to determine the cell of origin of the more common anaplastic tumors. Of ten 1-month-old mice, five had focal areas of hyperproliferative anaplastic neuroepithelium adjacent to the choroid plexus (see Figure 1D, arrowheads), and in one of the sections there was an enlarged bizarre mitotic figure (see Figure 1D, arrow). Three of ten 2.5-month-old mice had microscopic foci of hyperproliferative anaplastic neuroepithelium similar to the changes in 1-month-old animals. Two animals evidenced microscopic anaplastic tumors, not apparent grossly, arising posterior to the cerebral hemispheres, in a location similar to that of the more advanced lesions (Figure 1E). Of ten 4-month-

old asymptomatic animals, two also had microscopic anaplastic tumors. These data are consistent with the derivation of these anaplastic tumors from the transformation of primitive subependymal neuroepithelial cells.

Transgene Copy Number and Expression

Southern blotting was performed to determine the state of the transgene and its copy number in tumor and normal tissues. Transgene copy number in both tumor and normal tissues was estimated to be 4-5, arranged in a head-to-tail tandem array (data not shown). Digestion of genomic DNA and plasmid reconstructions with the isochizomers *Hpa*II and *Msp*I confirmed the transgene copy number of 4-5 and additionally showed heterogeneous methylation in all tissues, with no evidence of extensive demethylation in the tumor (Figure 2).

The extent of transgene expression was assessed using RNA PCR to detect transcription in an extensive distribution of tissues in an animal with an anaplastic tumor. Despite the potential for diffuse expression with the use of the β-actin promoter, expression was confined to the tumor. There were two

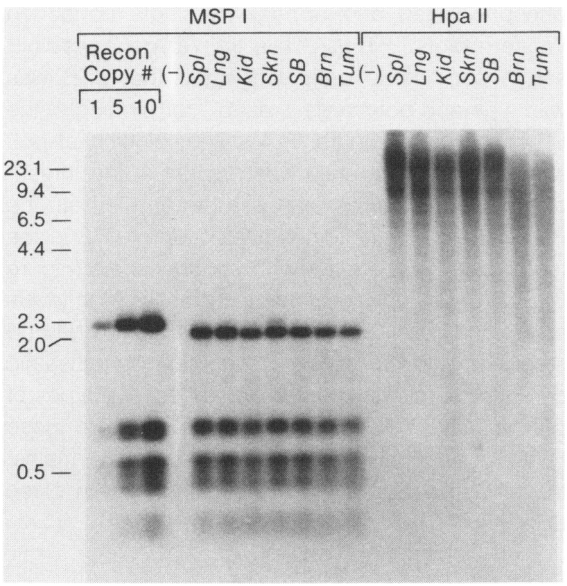

Figure 2. *A Southern blot of transgenic mouse genomic high-molecular-weight DNA digested with the isoschizomers MspI and HpaII. In the first three lanes are reconstructions with plasmid DNA, p1321, equivalent to 1, 5, and 10 copies of the transgene. - lane, DNA from a nontransgenic C57Bl/6J mouse. The remaining lanes are: Spl, spleen; lng, lung; kid, kidney; skn, skin; SB, small bowel; brn, uninvolved brain; tum, anaplastic brain tumor from a transgenic animal. The restriction pattern of tumor DNA is similar to those of normal tissues. The transgene copy number is 3-4. The transgene is predominantly methylated to a similar extent in tumor and normal tissues.*

bands present in the reverse transcriptase lane, a faint 431-bp band and a predominant 246-bp fragment (Figure 3). The former is consistent with full-length E6 mRNA, while the latter is due to the formation of a spliced E6* mRNA. E6* mRNA is also the predominant transcript found in HPV16- and 18-associated cervical cancers.[20] Transcription of both E6 and E7 mRNA in the tumor-associated HPVs is initiated from the P_{97} promoter, and E7 mRNA is produced from a E6*/E7 transcript which allows efficient translational initiation at the internal E7 ATG.[20] Northern analysis (data not shown) also demonstrated two HPV mRNA transcripts in tumor tissue.

HPV E7 Protein Expression in Tumor and Normal Tissue

The next experiments investigated whether the HPV E7 protein could be detected. (Antibodies of adequate affinity and specificity for detection of the E6 oncoprotein in both tissues and cultured cells are currently not available.) Tissue extracts from spleen and anaplastic tumors of two transgenic animals were immunoprecipitated using a polyclonal HPV16 E7 antibody and subjected to SDS-polyacrylamide gel electrophoresis, and after electrophoretic transfer the polyvinyldifluoride membranes were probed with an E7 monoclonal antibody. The E7 protein was detected in the tumors but not the spleens of both animals (Figure 4A).

Tumor Cell Lines

In order to further characterize the cellular phenotype of the two tumor types, cell lines from one of the anaplastic carcinomas (Br7 cell line) and a choroid plexus carcinoma (Br3 cell line) were derived. The appearance and growth of these two lines were markedly different. The Br7 line rapidly grew in suspension as clumps of cells that were nonadherent to either ordinary or treated plastic or to any of a number of artificial matrices. The Br3 cell line was adherent to ordinary plastic and formed sheets of epithelioid cells which were difficult to passage and grew very slowly. After passage 15 these cells became very sparse and were unable to be passaged further. Extracts of each of these primary cell lines (Br7 passage number 10, Br3 passage number 5) were prepared along with a HPV-negative mouse lymphoma cell line (NS-1) and a human cervical carcinoma cell line (CaSki) known to carry several hundred copies of integrated HPV16 DNA. The HPV16 E7 protein was present in

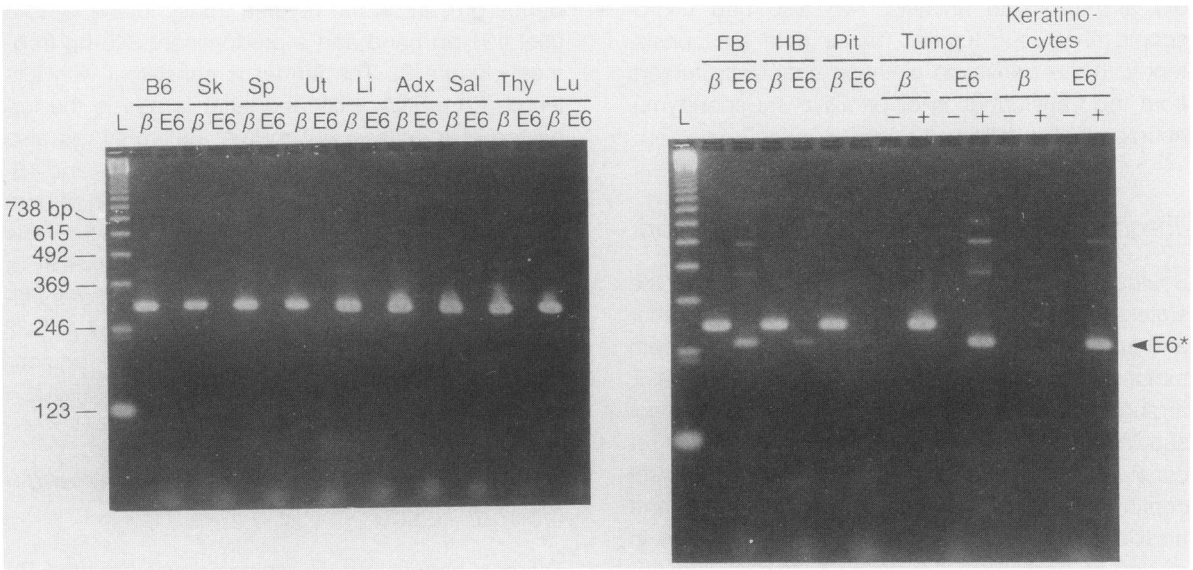

Figure 3. *An ethidium bromide-stained gel of a RNA-PCR tissue expression survey. RNA (1 μg) was reversed transcribed, and separate aliquots of the cDNA were amplified using primers specific for β_2-microglobulin (β) or the full length (E6) or spliced (E6*) HVP16 ORF. B6, spleen RNA from a C57Bl/6J nontransgenic animal; Sk, skin; Sp, spleen; Ut, uterus; Li, liver; Adx, adrenal; Sal, salivary gland; Thy, thymus; Lu, lung; FB, forebrain (coronal section of brain rostral to the superior colliculus); HB, hindbrain (coronal section of the brain caudal to FB); pit, pituitary; tumor, anaplastic carcinoma; keratinocytes, human foreskin keratinocytes transformed with p1321. The − and + lanes, respectively, are without and with reverse transcriptase to control for DNA contamination of the RNA. While the 302-bp cDNA fragment of β_2-microglobulin is present in all tissues only "normal" brain and tumor display both the 431-bp full-length E6 fragment and the 246-bp E6* fragment.*

both the Br7 and the Br3 cell lines in addition to the CaSki cells (Figure 4B).

To assess tumorigenicity, 2.5×10^6 cells from the Br7 cell line were injected either subcutaneously or intraperitoneally into athymic mice. Nine of nine subcutaneous and five of five intraperitoneal injections produced tumors in these hosts. The subcutaneous tumors reached a size of 2 cm in diameter in 2 weeks. Intraperitoneal injections produced ascites and multiple 1-cm intra-abdominal masses 4 weeks after injection. Due to the slow growth of the Br3 cell line and its difficulty in passaging, tumorigenicity experiments were not performed with these cells.

Characterization of the Status of Cellular Tumor Suppressor Genes

The E7 and E6 oncoproteins of HPV16 are known to bind to the retinoblastoma gene product (pRB) and p53, respectively. In order to determine whether the tumor cells had undergone independent mutations in these tumor suppressor genes during tumorigenesis, SDS-polyacrylamide gel electrophoresis and Western blotting were performed on cell extracts from both cell lines. pRB was present in both Br7 and Br3 cell lines. In addition, both cell lines exhibited multiple bands from M_r 105 to M_r 110, consistent with the presence of multiple phosphorylated forms of pRB. While point mutations in *Rb* can result in detectable protein, these mutant proteins are not phosphorylated and appear as single bands on Western blots.[10] Thus, while the *Rb* gene was not sequenced, these data suggested that pRB was wild type and potentially available for binding to the E7 oncoprotein (Figure 5). Western blotting of p53 showed that the protein was present at the threshold of detection in normal cells (nf) and in both the Br3 and the Br7 cell lines. Detection of p53 in the tumor cell lines required two separate gels (Figure 6). The βTC-3 cell line, which expresses high levels of simian virus 40 (SV40) T antigen that binds and stabilizes wild type p53 in oligomeric cytoplasmic complexes, mimics the behavior of several types of p53 mutants.[21] The very low levels of p53 in these HPV16 neuroepithelial cell lines is evidence against the presence of this type of p53 mutation in these cells.

Discussion

This report demonstrates that expression of the HPV16 E6/7 ORFs can induce both anaplastic and well-differentiated neuroepithelial tumors in transgenic animals. A previous report has documented the appearance of seminomas, at 7 months of age, in three lines of mice with HPV16 E6/E7 genes reg-

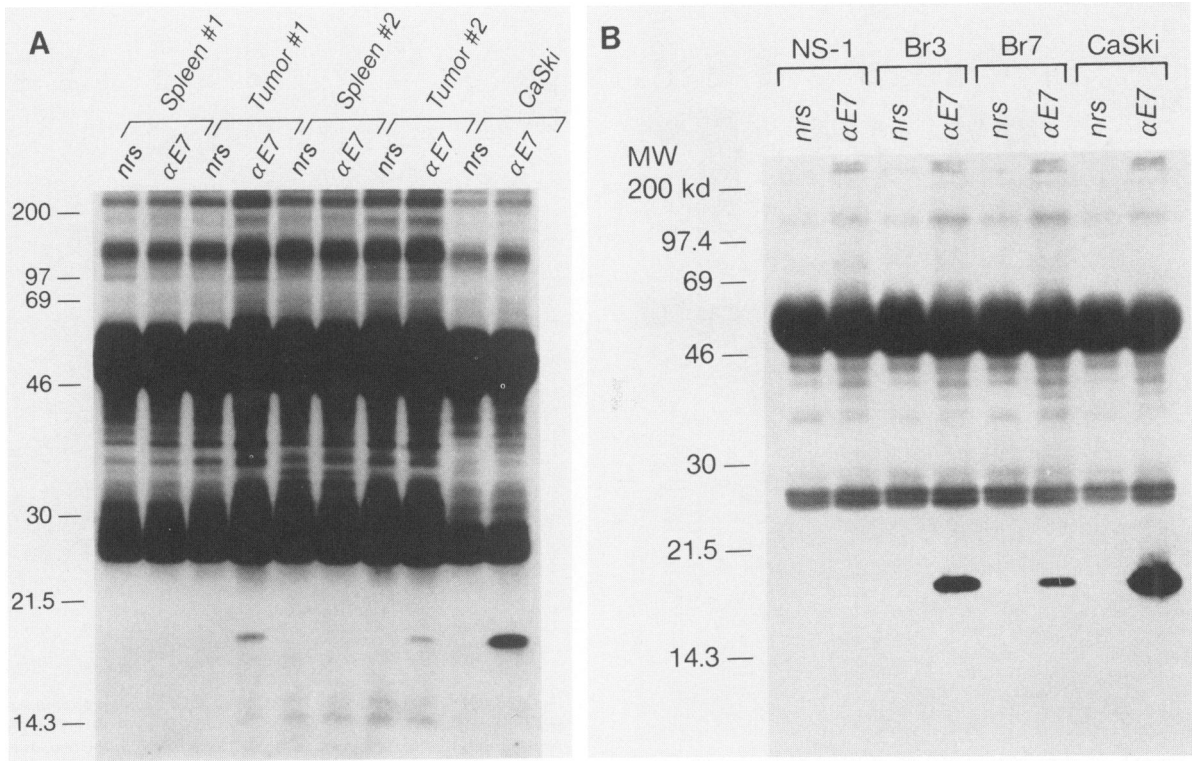

Figure 4. *Western blots of immunoprecipitated extracts from normal and tumor tissues* (**A**) *and control and tumor cell lines* (**B**). *In* **A** *both tumors display a specific 18-kd band that is present in CaSki tumors consistent with HPV16 E7 protein.*[5] *The CaSki tumors were grown in nude mice following the subcutaneous injection of 2.5 × 10*^{6} *CaSki cells. In* **B** *both tumor cell lines Br3 and Br7 contain the specific E7 band. Br3 cells are epithelioid and derived from a choroid plexus carcinoma. Br7 cells are grown as nonadherent clumps in suspension and are derived from an anaplastic neuroepithelial tumor. NS-1 cells are derived from a HPV-negative mouse lymphoma cell line. CaSki cells contain ~200–400 integrated copies of HPV16 DNA.*[2]

ulated by the mouse mammary tumor virus promoter.[15] In this study anaplastic tumors rapidly developed without a long period of latency. Locally invasive primitive tumors appeared at 2.5 months, with nascent abnormal hyperplastic cells evident as early as 1 month. HPV16 RNA and E7 oncoprotein expression was documented in both tumor tissue and cell lines derived from these tumors. Both pRB and p53 were shown to be present, with characteristics indicating that they are wild type in these tumor cell lines. These data suggest that tumorigenesis in this model was not due to independent mutations in these critical host tumor suppressor genes. While only one line of animals was established, the fact that the phenotype arose early, in heterozygotes, and that expression of both E6 mRNA and E7 protein was demonstrated in the tumors strongly suggests that tumorigenesis was related to the expression of the transgenes, rather than a random effect of integration.

Both of the tumor types seen in this model, anaplastic neuroepithelial and choroid plexus carcinomas, have been observed in previous work with mice transgenic with constructs containing the SV40 early region regulated by either its own enhancer/promoter or a long terminal repeat from the Moloney sarcoma virus.[22–25] Additional work using the lymphotrophic papovavirus and its regulatory region has shown similar brain tumors.[26] In contrast to these previous reports, the choroid plexus tumors seen in these β-actin HPV16 E6/7 animals were unifocal and more aggressive, resembling the grade IV tumors reported by Van Dyke.[24] The most common tumor type seen in this study, primitive neuroepithelial carcinomas, resembled both the anaplastic carcinomas reported by Van Dyke[24] and the primitive neuroectodermal carcinomas/pinealomas reported by Theuring.[25] Both of these groups used DNA encoding SV40 T antigen as the open reading frame for their transgenes. The neuroepithelial tumors in human β-actin HPV16 E6/E7 mice frequently invaded and destroyed adjacent tissue and spread for considerable distances along the inner ventricular surface. The primary cell line derived from this type of tumor was also highly tumorigenic in nude mice. These

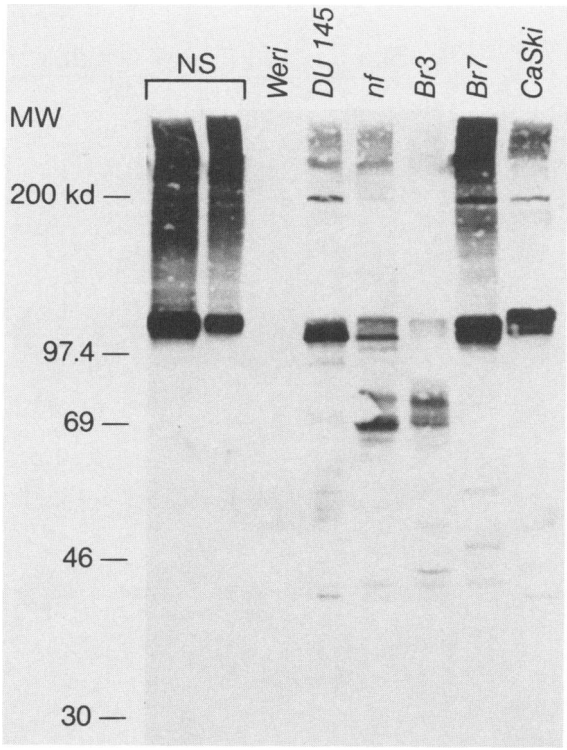

Figure 5. *Western blots of 100 μg of total cellular protein extract probed with a monoclonal anti-retinoblastoma antibody. Both HPV cell lines Br3 and Br7 display a p105–110 protein with multiple bands consistent with the multiple phosphorylated forms of the retinoblastoma protein. NS-1 cells are a murine lymphoma known to overexpress pRB, and 100 and 50 μg of protein extract were run in these lanes as positive controls. WERI-1 cells are from a human retinoblastoma known to have a deleted Rb gene. DU145 cells are from a prostatic cell line that produces a mutant truncated Rb protein. nf, normal primary fibroblasts derived from a neonatal nontransgenic mouse.*

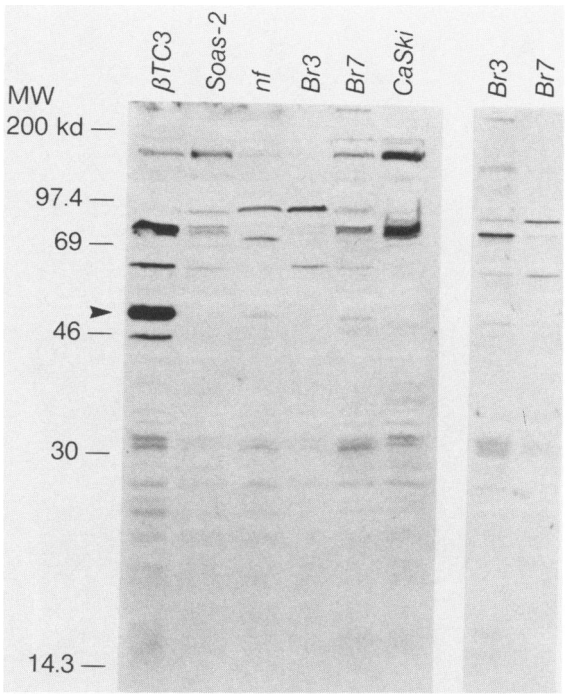

Figure 6. *Two Western blots of 200 μg of total cellular protein extracts probed with a anti-p53 monoclonal antibody. In the gel on the left a faint 53-kd band is seen in the Br7 lane, while in the two lanes on the right (cut from a separate gel) a similar band is seen in the Br3 lane. In both blots the level of p53 expression in the tumor cell lines is at the threshold of detection and similar to that seen in primary passage normal dermal mouse fibroblasts (nf). βTC-3 cells are a line derived from islet cell tumors arising in mice transgenic with a rat insulin I–promoted SV40 T antigen construct.*

data suggest either that the E6/E7 oncogenes were able to provoke a more aggressive tumorigenic phenotype than SV40 T antigen or that the neuroepithelial cells that were stochastically permissive for transgene expression in this model were more sensitive to the induction of a more virulent tumorigenic cell. Of note was the apparent difficulty in defining a preneoplastic phase. Even early brain dissection prior to the onset of overt disease showed either normal tissue or the initial onset of a nascent focus of anaplastic neuroepithelial cells containing a bizarre mitotic figure. These results again are in contrast to both Van Dyke's and Theuring's data, where a definite preneoplastic hyperproliferative phase in either the choroid plexus or the pineal gland could be dem-onstrated in young mice.[24,25] Also, the rapid progression of malignancy in the β-actin HPV16 E6/7 transgenic animals contrasts with human HPV-associated cervical cancer, where a long disease interval and discrete multistep progression exist.[1] The difficulty in identifying multistep progression in this model compared to either SV40 transgenic animals or human cervical cancers could be due to the target cell undergoing transformation. In these β-actin HPV16 E6/E7 transgenic animals the most common tumors appear to arise from a primitive neuroepithelial cell, compared to differentiated choroid plexus cells, and pineal gland in SV40 transgenic mice or human cervical epithelial cells.[1,22,24,25]

A common function shared by the DNA tumor viruses, SV40, adenovirus, and human papillomavirus that may induce proliferation and predispose to subsequent malignancy is the binding of virally encoded proteins to host cellular tumor suppressor gene products. A number of studies have demonstrated the binding of HPV16 E6 and E7 oncoproteins with p53 and pRB, respectively.[11–14] E6 has been shown to accelerate the destruction of p53 by the ubiquitin pathway, while E7 presumably sequesters pRB and may prevent complex formation between RB and host proteins involved in host transcriptional and cell cycle activation.[10,12,14] This

work has predicted that subsequent mutation in these host tumor suppressor genes would have no selective advantage in HPV-associated carcinogenesis. In support of this assumption, Scheffner et al[27] have shown in cervical carcinoma cell lines that contain HPV DNA that the *p53* and *Rb* genes were wild type, while cell lines with undetectable HPV contained point mutations in *p53*, and deletions of exons or splice junctions in *Rb*. In liver or choroid plexus tumors arising in transgenic mice expressing SV40 T antigen, *p53* has also been shown to be wild type.[28] In the present study of transgenic HPV carcinogenesis, while the *Rb* and *p53* genes were not sequenced, the Western analysis suggests that both gene products remain wild type and that tumorigenesis in this transgenic model also can occur without independent mutation in these two host tumor suppressor genes. In addition, the detection of p53 protein in the HPV primary transgenic cell lines may seem paradoxical given its proposed accelerated destruction. Both Scheffner et al[27] and more recently Lechner et al[29] have shown that p53 levels are generally low but detectable in HPV-containing cervical carcinoma cell lines. Together these data suggest that HPV E6 potentially targets a only subset of p53 for destruction *in vivo*.

Since HPV does not normally involve the brain, what is the relevance of this transgenic mouse model to human brain carcinogenesis? A number of studies have demonstrated allelic loss or point mutations of tumor suppressor genes in human brain carcinomas.[30] Point mutations or deletions in *p53* are particularly common in human gliomas, primitive neuroectodermal carcinomas, and ependymomas.[31–34] In the congenital Li-Fraumeni syndrome, where *p53* is mutant in the germ line and effected siblings die prematurely from single or multiple cancers, the third most common tumors are brain carcinomas.[35] Although less frequently reported, *Rb* mutations have been found in 20% of gliomas.[36] Thus the relevance of this HPV transgenic mouse model of brain carcinoma is the ability of the viral oncoproteins to bind and potentially inactivate either or both p53 and pRB. The functional inactivation of these two tumor suppressor gene products mimics a frequently reported molecular event in malignancies of the human brain.

In summary, this report demonstrates that the oncoproteins of HPV16 can induce three types of neuroepithelial brain carcinomas, which appear early and frequently, in transgenic mice. Presumably tumorigenesis in this system is induced via sequestration or inactivation of both pRB and p53. It would be interesting to determine whether neuroepithelial tumorigenesis can be induced with either the HPV16 E6 or the E7 ORFs alone. Transgenic mice could be made with either of these ORFs regulatedby the human β-actin, or other promoter/enhancers known to target neuroepithelial tissues.[25,37] An unrelated but obvious question is what would be the effect of targeting the expression of HPV16 oncogenes to a tissue more closely related to the natural site of HPV16-associated pathogenesis, ie, squamous epithelium? Using promoters expressed in keratinocytes,[38] it may be possible to induce multistep carcinogenesis in an epithelium with a defined architecture that would lend itself to a phenotype-based investigation of additional molecules that facilitate malignant conversion. The accessibility of skin would allow frequent observation, biopsy, and additional treatments, promoters, or initiators, which may potentially induce or enhance tumorigenesis.

Acknowledgments

The authors thank Dr. Larry Kedes for the gift of the human β-actin expression vectors pHβAPr-1 and Phβapr-3-neo, Dr. Denise Galloway for the gift of the polyclonal HPV16 E7 antibody, and Lena Delacio for technical assistance.

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