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Heritable Formation of Neuroectodermal Tumor in Transgenic Mice Carrying the Combined E1 Region Gene of Adenovirus Type 12 With the Deregulated Human Renin Promoter

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Abstract Adenovirus early 1 (E1) region gene products, including E1A and E1B, are required for transcriptional regulation of viral and cellular promoters in infected and transfected culture cells and for transformation of primary rodent cells. Here, we established a line of transgenic mice carrying the E1 region gene of human adenovirus type 12 under the control of the human renin promoter, in which a neuroectodermal tumor derived from retroperitoneal, olfactory, and/or pelvic regions was heritably developed with varying degrees of incidence and the phenotype was successfully passed through six generations. The transgenes were located in the region E2-E3 bands of chromosome 7 with which no genetic linkage to neuroectodermal tumors was previously demonstrated, and expressed only in the tumors but not in another tissue examined. Notably, in addition to the expression of a neural marker gene N-CAM, the three nuclear oncogenes, c-, L-, and N-*myc*, were coexpressed in the tumors. These results suggest that E1A and E1B are cooperatively involved in the heritable formation of neuroectodermal tumors associated with co-expression of the three sets of *myc* family genes.

Key words: adenovirus early 1, E1 region gene, human renin promoter, neuroectodermal tumor, *myc* genes

Human adenoviruses (Ad) are double-stranded DNA viruses, some of which are capable of inducing neurogenic tumors in the peripheral and central nervous systems of rodents [reviewed in Ogawa, 1989a,b]. The discovery by Trentin et al. [1962] of the oncogenic potential Ad type 12 (Ad12) in Syrian hamsters provided the first evidence that a virus of human origin can induce a highly malignant neoplasm. An interesting aspect of Ad is that their oncogenic potential varies [reviewed in Flint, 1984]. For example, earlier inoculation experiments have indicated that Ad12 is strongly oncogenic and induces tumors at high frequency, while Ad5 is nononcogenic. Virus or isolated DNA of all serotypes, however, can transform rodent cells in vitro. The tumorigenicity of the resulting transformed cells in nude mice and syngeneic rats reflects the oncogenic potential of the species used for transformation, Ad12-transformed cells being more oncogenic than Ad5-transformed cells [Mak et al., 1979; Gallimore and Panaskeva, 1980; Van den Elsen et al., 1982].

The transforming activity of the Ad viral DNA has been mapped within the early 1 (E1) region sequences [Bernards et al., 1983; Schrier et al., 1983], which consist of two transcriptional units, E1A and E1B [reviewed in Berk, 1986]. The E1A products alone are sufficient for induction of DNA synthesis [Kaczmarek et al., 1986; Stabel et al., 1985], modulation of cellular and virus transcription [Berk, 1986; Shenk and Flink, 1991], and cellular immortalization in vitro [Houweling et al., 1980; Ruley, 1983], but the coexpression of E1B or *ras* oncogenes in combination with E1A is required for fully and highly efficient oncogenic transformation of primary cultured rodent cells [Ruley, 1983; Shenk and

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Flink, 1991]. Although a large number of investigations concerning the Ad12 E1 region genes reported the effects of those products on in vitro transformation, there is still less information available upon the in vivo action of multifunctional E1A and E1B gene products when these genes are chromosomally integrated in the living animals.

In the present study, we have investigated an in vivo effect of Ad12 E1A and E1B genes on cellular transformation in transgenic mice. To deregulate the Ad12 E1 region genes, we have chosen a 3-kb upstream fragment of the human renin gene, which directed expression of the 12-kb renin-coding region mainly to the kidney in transgenic mice [Fukamizu et al., 1989, 1991a,b, 1993], because the original prototype of human Ad12 was isolated in primary human embryonic kidney cultures from a child [Kibrick et al., 1955, 1957]. Contrary to our initial expectation, the transgenic mice carrying the Ad12 E1A and E1B genes heritably developed neuroectodermal tumors arising from the olfactory, retroperitoneal, and/or pelvic regions with varying degrees of incidence. The transgene expression is limited to the tumors, where the three sets of *myc* genes, c-, L-, and N-*myc*, are coexpressed. This unexpected results, however, may reflect the high affinity of Ad12 for cells in the peripheral nervous system.

MATERIALS AND METHODS

Construction of Fusion Transgene and Production of Transgenic Mice

A unique XhoI site of pMTVAd12 [Koike et al., 1989], in which the long terminal repeat (LTR) of mouse mammary tumor virus (MMTV) is fused to the complete Ad12 E1 region genes, including those of E1A and E1B, was digested with XhoI and converted to the BglII site. The 5-kb BglII-EcoRI DNA fragment containing Ad E1 region genes was isolated from the resulting plasmid and cloned into the BglII-EcoRI sites of pUC19 to make pE1AB/G. The 3-kb BamHI DNA fragment containing the 5′-flanking region of the human renin gene was excised from pUChRNcat30 [Fukamizu et al., 1991b] and ligated into the BglII site of pE1AB/G to construct pUChRNE1AB.

For microinjection into fertilized eggs, pUChRNE1AB was cleaved with BamHI. The 8-kb insert DNA was isolated by sucrose gradient centrifugation to remove the vector sequences and dialyzed against 10 mM Tris-HCl buffer (pH 7.5) and 1 mM EDTA. The DNA was used directly for microinjection in a concentration of 4 μg/ml and about 1,000 copies/embryo. One-cell zygotes fertilized in vitro were obtained from C57BL/6 mice, and outbred CD-1 females were used as the pseudopregnant recipients. The transgenic procedure used was essentially as described [Hogan et al., 1986].

Southern Blot Analysis

Genomic DNA obtained from mouse tails and tumors was digested with HindIII, electrophoresed on a 0.7% agarose gel, and transferred to GeneScreen Plus membrane (DuPont-New England Nuclear, Boston, MA). Filters were hybridized to the ^{32}P-labeled 1.4-kb HindIII DNA fragment containing a portion of the E1B gene, at 65°C for 16 h and washed twice with 2 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) at room temperature for 5 min, twice with 2 × SSC/1% SDS at 65°C for 30 min, and twice with 0.1 × SSC at room temperature for 30 min.

Chromosome Preparation and In Situ Hybridization

R- and Q-banded chromosomes were prepared, and fluorescence in situ hybridization (FISH) was performed as described by Matsuda et al. [1992]. The 8-kb DNA fragment inserted in pUC19 was used as the biotinylated probe. The chromosome slides were hardened at 65°C for 2 h, denatured at 70°C in 70% formamide in 2 × SSC, and dehydrated in 70%, 85% and 100% ethanol series at 4°C. The probe was labelled by nick translation with biotin 16-dUTP (Boehringer Mannheim) following the manufacturer's protocol, ethanol-precipitated with salmon sperm DNA and *E. coli* tRNA, and then denatured at 75°C in 100% formamide. The denatured probe was mixed with an equal volume of hybridization solution to make final concentration of 50% formamide, 2 × SSC, 10% dextran sulfate, and 1 mg/ml BSA (Sigma). A twenty microliter mixture containing 250 ng of the biotinylated DNA probe was put on the denatured slides, covered with parafilm, and incubated overnight at 37°C. Slides were washed for 20 min in 50% formamide in 2 × SSC at 37°C, and in 2 × SSC and 1 × SSC for 20 min each at room temperature. After rinsing the slides in 4 × SSC, the slides were incubated under coverslip with avidin-conjugated fluorescein isothiocyanate (vector) at a 1:500 dilution in 1% BSA/4 × SSC for 1 h at

37°C. The slides were washed with 4 × SSC, 0.1% Nonidet P-40 in 4 × SSC, and 4 × SSC for 10 min each on the shaker. After draining the excess liquid from the slides, they were stained with 1.0 μg/ml propidium iodide. Excitation at wave length 450–490 nm (Nikon filter set B-2A) and near 365 nm (UV-2A) was used for observation. Kodak Ektachrome ASA100 films were used for microphotography.

Histopathological Analysis

Tissues for histopathological examinations were obtained from the tumors at autopsy. For light microscopy, the tissues were fixed in 10% buffered formalin and embedded in paraffin. Deparaffinized tissue sections were stained with hematoxylin-eosin (HE), periodic acid-Schiff's solution (PAS), and alcian blue. For electron microscopy, the tissues were minced into small blocks and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (PB) of pH 7.4 at 4°C. The tissue blocks were postfixed with 1% osmium tetraoxide in PB for 1 h, dehydrated in graded alcohols, and embedded in Epon 812. Ultrathin sections were stained with lead citrate and uranyl, and observed under a JEM 1200 electron microscope (JEOL, Tokyo, Japan).

Northern and Western Blot Analyses

Total RNA was denatured with 1 M glyoxal and 50% dimethylsulfoxide, electrophoresed on a 1.2% agarose gel, and transferred to GeneScreen Plus membrane. Filters were hybridized to the ^{32}P-labeled DNA probes as described below, at 60°C for 16 h and washed twice with 2 × SSC at room temperature for 5 min, twice with 2 × SSC/1% SDS at 60°C for 30 min, and twice with 0.1 × SSC at room temperature for 30 min. The used DNA probes are 783-bp BamHI/BglII DNA fragment for N-CAM from pDW3 [Barthels et al., 1987], 415-bp KpnI/ScaI DNA fragment for E1A or 724-bp AccI/HindIII DNA fragment for E1B from pMTVAd12 [Koike et al., 1989], 987-bp PstI DNA fragment for N-*myc* from pNc-1 [Kato et al., 1990], 404-bp BamHI/XhoI DNA fragment for c-*myc* from pBR322myc [Stanton et al., 1983], 806-bp SacI DNA fragment for L-*myc* from pL-Myc [Legouy et al., 1987].

Each tissue from transgenic and nontransgenic mice was homogenized in 4 volumes of phosphate-buffered saline (PBS) (pH 7.2) containing 5 mM EDTA and 5 mM PMSF, followed by centrifugation at 100,000*g* for 60 min at 4°C. The concentration of supernatants was determined using bovine serum albumin (BSA) as a standard [Bradford, 1976] and 20 μg of proteins were subjected to the electrophoresis of polyacrylamide gel containing 10% sodium dodecyl sulfate (SDS) as described previously [Laemmli, 1970]. Proteins from SDS gel were transferred electrophoretically at 200 mA for 20 min to Immobilon membrane (Millipore, Bedford, MA) in a Zaltblot electrophoretic transfer unit (Pharmacia, LKB Biotechnology, Uppsala, Sweden). Transferred proteins were immunoblotted with an anti-serum against Ad12 E1A [Shiroki et al., 1992] (dilution 1:1,000 in 20 mM Tris-HCl, pH 7.5, containing 500 mM NaCl and 1% BSA). The signal was generated and detected by using ECL Western blotting system (Amersham, Buckinghamshire, UK).

RESULTS

Production of Transgenic Mice

The 3-kb human renin promoter region was fused in front of the Ad12 E1A and E1B genes, the latter of which possessed its own natural promoter that contains transcriptionally important *cis*-acting DNA elements such as TATA and GC sequences (Fig. 1). By means of the first series of microinjection experiments, we have initially generated three transgenic mice carrying the Ad12 E1A and E1B (hRNE1AB) gene, all of which had the one copy of transgene. However, no progeny could be obtained from the founder mice because of their early death 1 month after birth. By the second series of experiments for microinjecting the 8.5-kb hRNE1AB gene (Fig. 1) into mouse fertilized eggs, we further tried to generate transgenic mice and succeeded in producing 43 offspring. DNA from tails of potentially transgenic mice was screened by Southern blot analysis using an E1B gene DNA probe. Of offspring produced, one female (F) and two males (M) (hRNE1AB 14F, 35M, and 36M) were shown to carry the 5, 1, and 10 copies of transgene, respectively (data not shown). Because hRNE1AB 36M developed a tumor arising from olfactory regions within 2 months after birth, the mouse was sacrificed and subjected to an in vitro fertilization experiment with oocytes obtained from a nontransgenic mouse of the inbred strains C57BL/6 to expand this lineage for further analysis. On the other hand, the two remaining founder mice appeared healthy until 20 months of age.

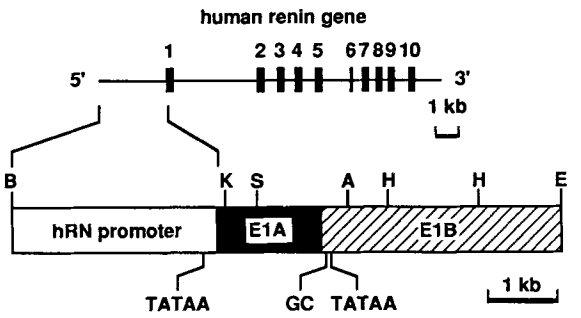

Fig. 1. Structure of the hRNE1AB transgene. **Top:** The human renin gene. The filled boxes and thin line represent the coding exons and the introns as well as flanking sequences, respectively. The coding exons were numbered. **Bottom:** The hRNE1AB gene. The open, filled, and hatched boxes represented the human renin promoter, the E1A gene, and the E1B gene, respectively. TATAA and GC represent the TATA and GC boxes, respectively. B, BamHI; K, KpnI; S, ScaI; A, AccI; H, HindIII; E, EcoRI.

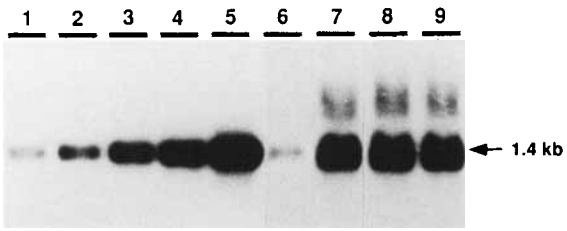

Fig. 2. Amplification of transgenes during transmission from founder to F1 generation. Southern blot analysis of transgenic mouse DNA. Five micrograms of genomic DNA were digested with HindIII and hybridized with the transgene probe. Lanes 1–5 contain copy markers 10, 20, 50, 100, and 200 copies, respectively. Lanes 6–9 contain genomic DNA isolated from hRNE1AB 36M, 102M, 203M, and 214M, respectively. The 1.4-kb position represents the HindIII genomic E1B fragment.

Germ-Line Transmission of Transgene

By Southern blot hybridization, we have analyzed hRNE1AB 36M lineage to learn whether the foreign gene sequences were transmissible to progeny, as evidence for integration in the germ line. Genomic DNA was digested with HindIII and subjected to Southern blot hybridization, using the 1.4-kb DNA fragment containing a portion of the E1B gene as a probe. In this strain, a major increase in copy number (from 10 to 200 copies) was clearly evident in hRNE1AB 102M, the only one transgenic F_1 progeny obtained (Fig. 2). This amplified copy number was successfully maintained from F_2 to F_6 generations, which were produced by mating hRNE1AB 102M with C57BL/6, suggesting a stable transmission of the transgene to progeny (data not shown). These results indicated the possibility that hRNE1AB 36M was a mosaic with the transgene sequences present in only a minority of the germ cells as well as in the somatic cells, but that the amplified copies of transgene from the F_1 generation were integrated at single chromosome sites.

In order to confirm the latter possibility, we determined where the transgene sequences were introduced into the transgenic mouse chromosome, using an F_3 progeny transgenic mouse (hRNE1AB 328M) that had yet to show signs of illness. For chromosomal assignment of the transgene, FISH was performed as described previously [Matsuda et al., 1992]. We determined the location of the transgene with R- and Q-banded chromosomes. The fluorescent signals were detected in the region E2-E3 bands of chromosome 7 (Fig. 3a and c). The signals were also observed on Q-banded chromosomes as light-blue spots because they were very bright and large (Fig. 3b and d), clearly demonstrating that the multiple copies of transgene were integrated at the single chromosomal site.

Pathological Analysis of Tumors

Autopsy revealed grossly visible tumors in 21 of 43 (about 49%) transgenic mice in the hRNE1AB 36M lineage. At 2 months of age, the founder mouse, hRNE1AB 36M, developed tumors arising from multiple sites, including the olfactory, retroperitoneal, and pelvic regions, whereas the tumors in hRNE1AB 102M were induced from the retroperitoneal and pelvic regions at 8 months of age. As summarized in Table I, among the progeny from F_2 to F_6 generations, these developed the olfactory, retroperitoneal, and/or pelvic tumors with varying degrees of incidence. On gross appearance, the tumors grew as large, round or lobulated, demarcated soft masses of red-gray to yellow-tan aspect on cut surface. Moribund hRNE1AB 205M, 219F, 211M, 314M, and 404F had loops of bowel distended by unknown cause, but no tumors in these mice were detected by either gross or microscopic examination (data not shown).

On histologic examination, all of the tumors arising from the olfactory, retroperitoneal, or pelvic regions exhibited tumor morphologies with malignant neuroectodermal properties. The neuroectodermal tumors were composed of closely packed sheets of lobules of small rounded cells containing darkly staining, round, or oval nuclei (Fig. 4A and B). These cells frequently formed tubular rosettes or glandular structures, which

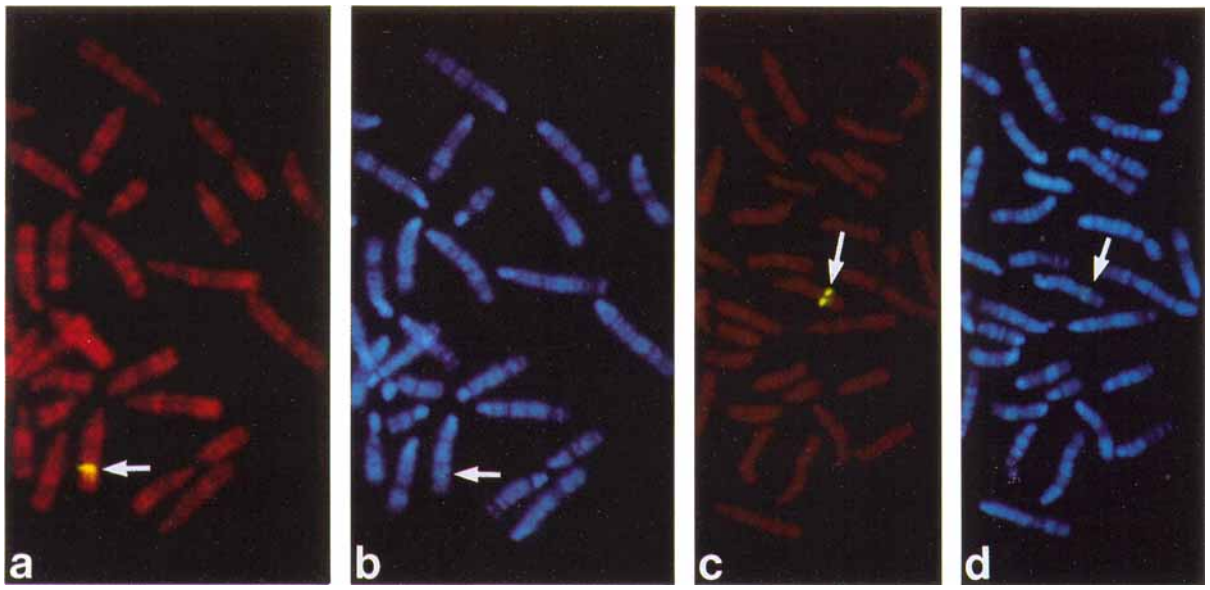

Fig. 3. Chromosomal localization of the transgene on R- (**a,c**) and Q-banded (**b,d**) chromosomes of the hRNE1AB line by FISH. Arrows indicate signals on E2-E3 bands of chromosome 7.

TABLE I. Neuroectodermal Tumors of hRNE1AB Transgenic Mice*

Mice	Sex	Age (mo)	Location
36	M	2	O, R, P
102	M	8	R, P
203	M	8	P
204	M	7	R, P
206	M	8	O
214	M	2	R, P
317	F	7	O
322	M	2	R
324	M	2	R
325	M	2	R, P
408	M	9	O, R, P
417	M	9	O, P
420	F	9	O
425	M	3	R
429	F	11	R
431	F	13	O
503	M	6	P
513	M	5	R
516	M	6	O
521	M	2	O, R
606	F	2	O, R

*M, male; F, female; O, olfactory region; R, retroperitoneal region; P, pelvic region.

mimicked a primitive neural tube and contained no mucinous material. By electron microscopy, the tumor cells showed the most striking ultrastructure with elongated cell processes that interdigitated with each other and contained dense core granules (Fig. 4C).

Molecular Property of the Neuroectodermal Tumors

In order to define the tissue specificity of transgene expression, total RNA was isolated from a variety of tissues, including kidney, liver, brain, and neuroectodermal tumor, and subjected to Northern hybridization analysis. As shown in Figure 5A, the transgenes, E1A and E1B, were expressed only in the tumors (lane 4). Western blot analysis also revealed the presence of Ad12 E1A protein in the tumor, but not in the liver (Fig. 5B). As the tumors expressed the E1A and E1B genes, presumably under the control of transcriptional regulatory sequences of the human renin gene, we anticipated that the tumors would also express endogenous renin. However, although the expression profiles of mouse renin gene in various tissues of transgenic mice, such as the kidney and brain, were almost identical to those found in the age-matched control mice, the renin mRNA was unable to be detected in the tumors (data not shown). These results indicated that the transgene expression is limited to the tumors and lacks its tissue specificity.

Several lines of evidence indicate that neural cell adhesion molecule (N-CAM) mRNA and its protein isoforms can be detected in different neuroectodermal tumors and normal brain tissues [Barbas et al., 1988; Garin-Chesa et al., 1991; Phimister et al., 1991]. To examine expression of the N-CAM gene in the neuroectodermal

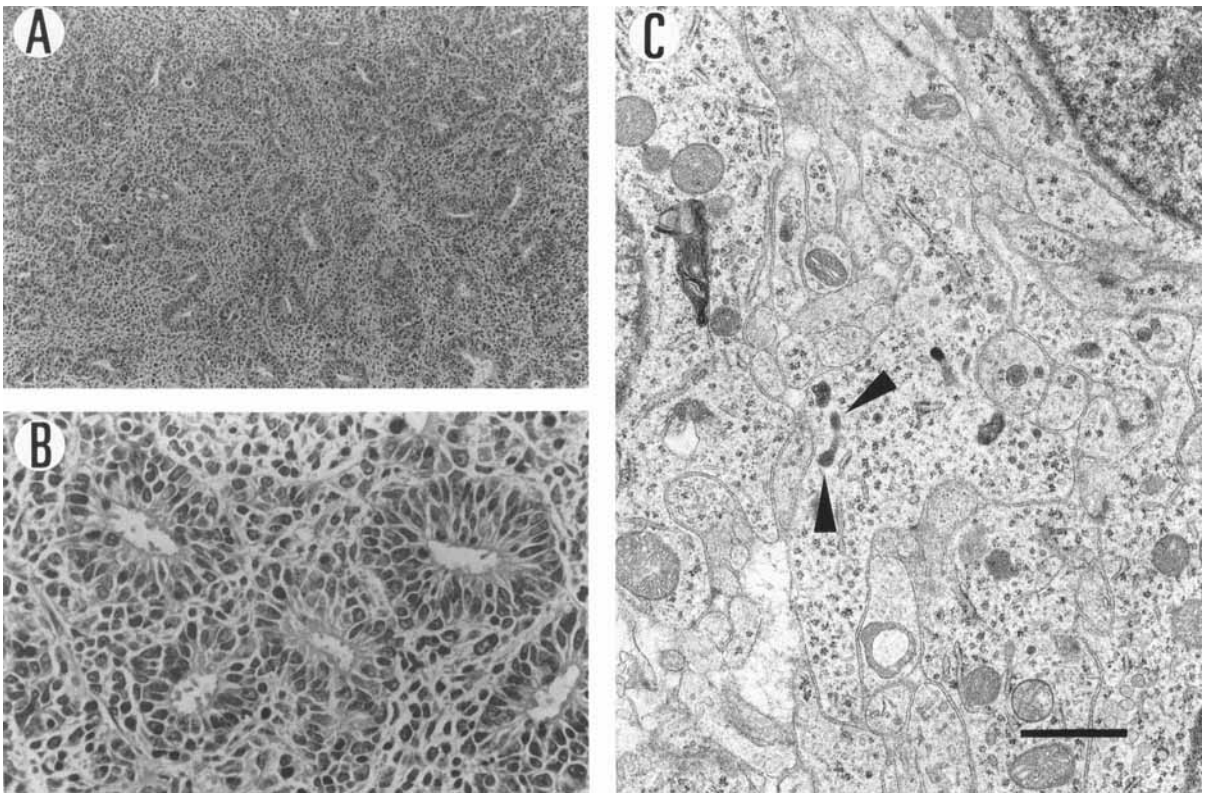

Fig. 4. Histopathological analysis of tumors. **A,B:** Microscopic section of the tumors stained with HE. ×25 (A) and ×100 (B). **C:** Electron micrograph of the tumor. The arrowheads indicate dense core (neurosecretory) granules. Scale bar, 1 μm.

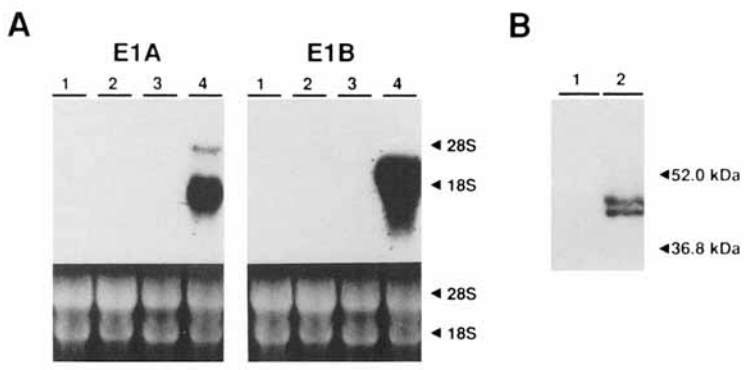

Fig. 5. Northern and Western blot analyses. **A:** Northern blot analysis of E1A and E1B genes. Twenty micrograms of total RNA isolated from the transgenic mouse liver (lane 1), kidney (lane 2), brain (lane 3), and tumor (lane 4) were used for blotting. Positions of the 28S and 18S rRNA species were indicated. The lower panels are UV photographs of the EtBr-stained gel prior to blotting. **B:** Western blot analysis of E1A protein. Each protein extract (20 μg) from liver (lane 1) and tumor (lane 2) was subjected to SDS-PAGE, performed with 10% polyacrylamide gel under reducing condition. After electrophoresis, the proteins were transferred to Immobilon membrane followed by immunoreaction with the anti-Ad12 E1A antibody.

tumors developed in transgenic mice carrying the Ad12 E1 region gene, total RNA was subjected to Northern hybridization. Although N-CAM mRNAs were undetectable in the control liver, the tumors produced multiple species of N-CAM mRNA at levels lower than those found in the mouse brain (Fig. 6A), confirming a neural origin of the tumors.

The *myc* family of nuclear proto-oncogenes, N-, c-, and L-*myc*, is well known to be activated in various tumors [Schrier and Peltenburg, 1993]. For example, in contrast to the c-*myc*

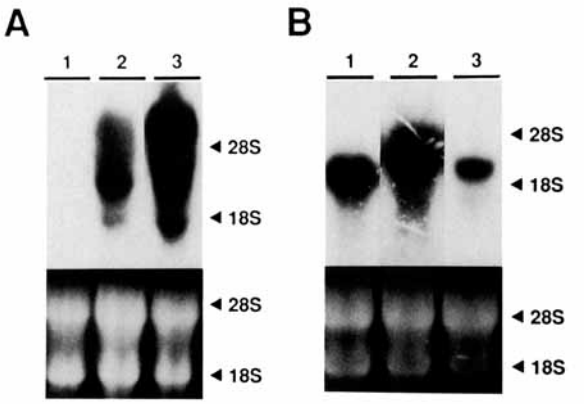

Fig. 6. Expression of N-CAM and *myc* family genes. **A:** Northern blot analysis of N-CAM gene. Five micrograms of total RNA isolated from the following tissues were used: lane 1, mouse liver; lane 2, mouse brain; lane 3, transgenic mouse tumor. **B:** Northern blot analysis of *myc* family genes. Twenty micrograms of total RNA isolated from the tumor were hybridized to the c-*myc* probe (lane 1), the L-*myc* probe (lane 2), or the N-*myc* probe (lane 3). Positions of the 28S and 18S rRNA species were indicated. The lower panels are uv photograph of the EtBr-stained gel prior to blotting.

gene activation in a wide variety of tumors, genomic amplification of N-*myc* and its consequently increased expression have been found in 30% primary neuroblastomas and also found in a number of other human tumors of neuroectodermal origin [Schwab et al., 1983; Kohl et al., 1984; Lee et al., 1984; Nisen et al., 1986]. Activation of L-*myc* has been implicated clearly only in human small cell lung carcinomas [Nau et al., 1985]. To explore expression of the N-*myc* gene as well as c- and L-*myc* genes in the newly developed neuroectodermal tumors of transgenic mice, Northern blots were performed using DNA probes that can specifically distinguish each *myc* gene. Surprisingly, Figure 6B demonstrated that the three sets of *myc* genes were coexpressed in the transgenic tumors. On the other hand, the copy numbers of three *myc* genes in the original tumors were not amplified as judged by Southern blot analysis (data not shown).

DISCUSSION

In the present study, we have established and characterized a line of transgenic mice that heritably develop tumors with pathologically neuroectodermal properties, arising mainly from the olfactory, retroperitoneal, and/or pelvic regions. The founder mouse, hRNE1AB 36M, carried the ten copies of the human renin-Ad12 E1A/E1B transgene, whereas all the progeny from the next generation to the sixth retained the 200 copies of the fusion gene. This increase in the copy numbers suggests that the founder was a mosaic that was heterozygous for the donor sequences in some cells while lacking these sequences in other cells. Furthermore, although the copy numbers were amplified during the course of propagation, FISH analysis using the F_3 transgenic progeny reveals that the transgene is integrated at the single sites of E2-E3 region on chromosome 7. These results indicate the stable germ-line transmission of the transgene to progeny.

Expression of the E1A gene fused immediately downstream to the human promoter sequences was not detected in any tissues examined, and limited to the developed neuroectodermal tumors of transgenic mice. The E1B gene under the control of its own native promoter that contains TATA and GC elements was also expressed only in the tumors, suggesting the *trans*-activation of E1B promoter by E1A. This observation is consistent with the fact that the E1B TATA and GC box regions respond to E1A *trans*-activation [Wu et al., 1987; Weintraub and Dean, 1992].

In 49% of our transgenic mice, gross tumors occurred at 2 to 13 months of age; the average age of appearance was approximately 6 months. The kinetics of induction of neuroectodermal tumors was not constant, even within the respective generations, but the regions where the tumors developed were confined to the olfactory, retroperitoneal, and/or pelvic spaces. Interestingly, a mouse model for retinoblastoma has previously been reported in which only 27% of the transgenic mice produced brain tumors, whereas 100% of transgenic mice produced retinal tumors [Windle et al., 1990]. This variation in the levels of tumor occurrence has been attributed to the effect of the integration site of the transgene on the levels, cell type, or onset of expression [Windle et al., 1990]. Thus, the variable incidence of neuroectodermal tumors arising from the multiple but limited sites in our transgenic mice is most likely due to the effect of integration site on the levels of expression of the transgene. Also, such a stochastic fashion of tumor appearance with variable latency seems to corroborate the notion that some additional events are necessary to induce the tumors. However, because clearly defined preneoplastic stages have been difficult to identify in our transgenic mice, it is not yet clear whether the process of

neuroectodermal transformation follows the same pattern of multistage tumorigenesis, with the requirement for one or more secondary events, as previously described in other cell types [Hanahan, 1985, 1989; Ornitz et al., 1987].

The limited expression of the *trans*-E1A and -E1B gene in our transgenic mice strongly implies the involvement of both viral oncogenes in the formation of neuroectodermal tumors. Since an earlier study indicated that high incidence of malignant tumors was produced by inoculating hamsters with Ad12 [Trentin et al., 1962], the effects of this oncogenic DNA virus upon the central and peripheral nervous systems of rodents have extensively been investigated. A large number of studies suggest that some neuronal primordia are particularly susceptible to Ad12 during a certain period of development [reviewed in Mukai, 1976]. Furthermore, it has been shown that E1A itself fails to induce transformation of primary rodent cells efficiently, which can be attributed to the induction of abortive transformation and cell death, although the E1A gene products interact with and perturb the function of key regulator of cell growth, such as the retinoblastoma protein [Whyte et al., 1988]. Several lines of recent in vitro evidence indicate that induction of apoptosis by E1A impedes transformation despite the ability of E1A to recruit cells to a proliferative state and that p53, a suppressor oncogene, mediates apoptosis by E1A [Rao et al., 1992; White et al., 1992], while the inhibition of p53 function by E1B is shown to block apoptosis and to lead to high-frequency transformation in cooperation with E1A [Rao et al., 1992; Debbas and White, 1993]. In the light of these in vivo and in vitro findings, an excess of Ad12 E1A protein in combination with E1B may confer a selective advantage for growth on neuronal progenitor cells by inducing proliferation and blocking apoptosis, resulting in the development of neuroectodermal tumors in transgenic mice.

The significance of when this malignant transformation begins remains to be established. Interestingly, however, we found the co-expression of c-, L-, and N-*myc* genes in the neuroectodermal tumors of transgenic mice. Therefore, this unexpected co-expression of the three sets of nuclear oncogenes may provide a good hint for the timing of tumor incidence. Expression of c-*myc* occurs in a variety of cell types and generally parallels a proliferative state, whereas expression of N- and L-*myc* in normal cells occurs at early stages of various cell lineages and is much more restricted than that of c-*myc* [Jakobovits et al., 1985; Zimmerman et al., 1986]. Nevertheless, it should be noted that the three sets of *myc* genes could transiently be co-expressed only at a short period during mouse fetal and postnatal development [Zimmerman et al., 1986]. Conceivably, a neuroectodermal cell with a marked propensity to Ad12 E1A and E1B might proliferate aggressively in the olfactory, retroperitoneal, and/or pelvic regions and begin to transform to the malignant tumors around at a fetal or postnatal stage that is characterized by the coexpression of the three *myc* family genes. To date, however, such coexpression has not been reported in transgenic mice that develop neuroectodermal tumors [Small et al., 1986; Aguzzi et al., 1990; Koike et al., 1990; Skalnik et al., 1991; Iwamoto et al., 1993].

In summary, the present study showed that transgenic mice carrying the human renin promoter-Ad12 E1A/E1B fusion gene heritably develop neuroectodermal tumors arising from the olfactory, retroperitoneal, and/or pelvic regions with a variable latency, in which the three sets of c-, L-, and N-*myc* genes are highly coexpressed. Our mouse model may be particularly useful in defining the molecular biological and biochemical basis underlying this type of human disorder. In addition, the isolation of a cell line derived from neuro-ectodermal tumors of the transgenic mice as a clonal source will provide a new tool for studying not only the relationship between E1 region products and apoptosis, but also the so poorly defined functions of *myc* family genes. Efforts are underway to establish such cell line.

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