Trilateral Tumors in Four Different Lines of Transgenic Mice Expressing SV40 T-Antigen

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Purpose. A line of transgenic mice containing the simian virus (SV) 40 T-antigen (T-ag) gene driven by the beta-luteinizing hormone (BLH) promoter developed bilateral retinoblastoma and primitive neuroectodermal tumors (PNET) of the midbrain. Midbrain tumors arose from the subependymal layer of the cerebral aqueduct. Bilateral ocular and brain tumors ("trilateral") were found in three other SV40 T-ag transgenic murine lines containing different promoters (murine interphotoreceptor retinoid-binding protein (IRBP), human IRBP, and alpha A-crystallin). To gain insight into the regulatory mechanisms involved in central nervous system tumorigenesis, the authors examined brain tumors from four lines of SV40 T-ag mice with different promoters.

Methods. Formalin-fixed brain tumors were examined from four lines of transgenic mice containing different promoters linked to the protein coding region of the enhancerless SV40 T-ag oncogene. Transgenes contained the following promoters: BLH, mouse 1.8-kb IRBP, human 1.3-kb IRBP, and alpha A-crystallin.

Results. Mice with a 1.8-kb IRBP promoter develop retinal photoreceptor and pineal tumors. Intracranial tumors arising from the subependymal layer of the third ventricle also were observed. Mice with a 1.3-kb IRBP promoter exhibit bilateral retinal PNET and PNET originating from the subependymal layer of the third ventricle. Mice with the alpha A-crystallin promoter exhibit bilateral lens tumors and PNET of the midbrain.

Conclusions. Ocular tumors in these mice may be ascribed to the promoter-driven, tissue-specific expression of SV40 T-ag. The common finding of PNET arising from the subependymal layer of the diencephalon is unlikely to be promoter related. These findings indicate that a regulatory region specific to the subependymal layer of the cerebral aqueduct and third ventricle resides in the structural region of the SV40 T-ag gene. Invest Ophthalmol Vis Sci. 1996;37:392–396.

In humans, the rare association of retinoblastoma with a similar primary intracranial midline neoplasm is known as "trilateral retinoblastoma."[1–4] We have described the murine counterpart of trilateral retinoblastoma occurring in transgenic mice containing the beta luteinizing hormone promoter (BLH) gene fused to the simian virus 40 (SV40) large T antigen (T-ag) oncogene.[5,6] These mice exhibited retinal-specific expression of SV40 T-ag, resulting from regulatory effects exerted by the transgene integration site, and they represent the first heritable model of retinoblastoma.[7] Although ocular tumors are observed in 100% of transgene-bearing mice, central nervous neoplasms occur at a lower rate (27%). Transgenic brain tumors are primitive neuroectodermal tumors (PNET) of the midbrain and arise from the subependymal cells of the cerebral aqueduct.[6]

Human intracranial trilateral retinoblastoma occurs in the region of the pineal or as a suprasellar or parasellar neoplasm.[3,4] The absence of primary pineal involvement in the BLH transgenic mouse model indi-

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cates that murine midbrain PNET represents a model for the suprasellar or parasellar form of human trilateral retinoblastoma.[6]

The development of central nervous system tumors in transgenic mice expressing T-ag is not unique to the BLH mouse model of retinoblastoma. Other lines of T-ag transgenic mice develop choroid plexus papillomas at 3 to 5 months of age.[8,9] The SV40 enhancer (72-bp repeat region) seems to direct expression of SV40 T-ag to the choroid plexus in the majority of transgenic mice. Choroid plexus tumors selectively express SV40 T-ag, a requirement for tumorigenesis in these animals. Transgenic mice with the JC virus T-ag under the control of the SV40 enhancer also develop choroid plexus tumors, providing direct evidence that SV40 viral regulatory regions play a critical role in determining the tissue specificity of viral oncogene expression.[10]

Substitution of tissue-specific promoter–enhancer elements for the SV40 enhancer redirects expression of T-ag to specific cell types and greatly reduces the incidence of choroid plexus tumors. For example, fusion of enhancerless SV40 T-ag genes (BglI site to the BamHI site of SV40 early region) to regulatory regions of elastase,[11] insulin,[12] and atrial natriuretic factor[13] result in transgenic mice with pancreatic acinar cell, pancreatic islet cell, and heart cell tumors, respectively.

Fusion of enhancerless SV40 T-ag to regulatory regions of various ocular-specific genes has resulted in the ocular-specific expression of SV40 T-ag with resultant ocular neoplasia. Transgenic mice carrying the murine alpha A-crystallin promoter fused to the enhancerless SV40 T-ag oncogene develop lens tumors.[14] Expression of SV40 T-ag under the control of a 1.3-kb human interphotoreceptor retinoid-binding protein (IRBP) causes bilateral retinal primitive neuroectodermal tumors.[15] In addition, a 1.8-kb murine IRBP promoter linked to the SV40 T-ag oncogeny produce transgenic mice with similar photoreceptor cell tumors.[16]

Interestingly, trilateral brain tumors also are seen in 100% of mice with the 1.3- and 1.8-kb IRBP promoters.[15,16] The precise origin of brain tumors was not established clearly in the 1.3-kb IRBP model. In the 1.8-kb IRBP promoter model, brain tumors were demonstrated to be pineal in origin. This finding is not unexpected because IRBP is expressed normally within the pineal gland. However, we have since identified a second primary intracranial tumor in the 1.8-kb IRBP model. In addition, mice with the alpha crystallin promoter were found to develop midline central nervous system tumors.

What is directing SV40 T-ag to the brain of these animals? Are these central nervous system tumors similar? We addressed these questions by examining brain tumors from transgenic mice carrying the alpha-crystallin, 1.3-, and 1.8-kb IRBP promoters fused to an enhancerless SV40 T-ag gene and compared them to the primitive neuroectodermal tumors found in mice with trilateral retinoblastoma.

MATERIALS AND METHODS

Four formalin-fixed, paraffin-embedded brain tumors occurring in 3-week-old trangenic mice carrying the 1.8-kb mouse IRBP promoter linked to the SV40 T-ag oncogene were examined.[16] The 1.8-kb IRBP T-ag transgene has been described[16] and contains the promoter region of the mouse IRBP gene (−1783 to +101) linked to an enhancerless–promoterless SV40 T-ag early region encoding large and small T-antigens.[16]

We examined formalin-fixed brain tumors from mice encoding the 1.3-kb promoter of the human IRBP gene fused to the 5′ end of the enhancerless–promoterless SV40 T-ag gene.[15] These mice have been described.[15]

We examined four formalin-fixed, paraffin-embedded brain tumors occurring in transgenic mice (3 to 5 months of age) carrying the murine alpha A-crystallin promoter fused to an enhancerless SV40 T-ag.[14] The alpha A-crystallin-SV40 T-ag construct has been described.[14] The 412-bp alpha crystallin sequence (−366 to +46) contains the promoter and cap site. The promoter was fused to an enhancerless–promoterless SV40 early region containing coding sequences for both large and small T-antigens and polyadenylation signals.[14] All animals were killed humanely in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

RESULTS

The 1.8-kb (murine IRBP promoter) IRBP T-ag mice demonstrated the development of retinal photoreceptor cell and pineal tumors by 2 weeks of age.[16] Previously described pineal tumors display Homer Wright rosettes.[16] Pineal tumors fill the transverse fissure of the cerebrum, spread through the pia mater, and by 5 weeks invade the outer molecular layer of the colliculus posterior and below the cerebral hemispheres.[16]

A second primary midline intracranial tumor can be identified at 3 weeks in the ventral portion of the third ventricle, at the level of the infundibulum hypothalami. This tumor is composed of lightly hyperchromatic monomorphous cells with scanty cytoplasm and round nuclei with sparse chromatin. Numerous mitoses are present, but there is no evidence of rosette formation or differentiation. Serial sections of four mouse brains demonstrate that the tumor originates

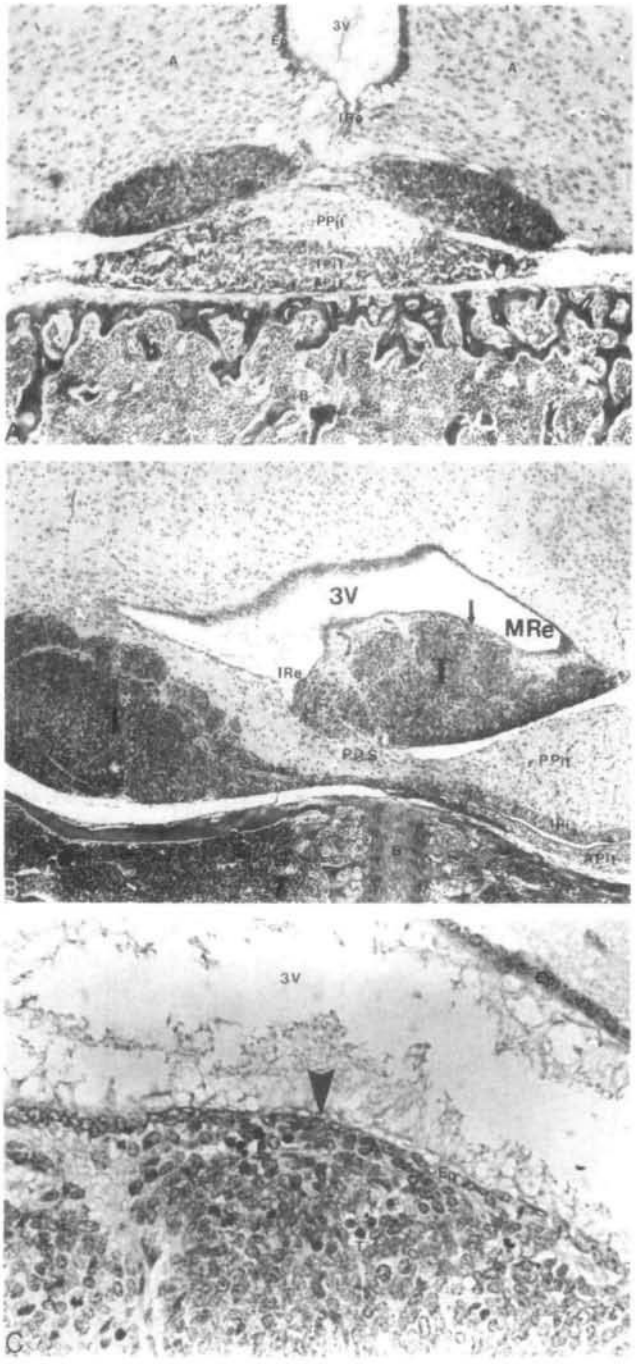

FIGURE 1. Simian virus 40 T-antigen mouse with a 1.8-kb murine interphotoreceptor retinoid-binding protein promoter develops retinal photoreceptor and pineal tumors. A second primary midline intracranial tumor is evident at 3 weeks of age. (**A**) Transverse and (**B**) sagittal sections demonstrate that this tumor (T) is located in the ventral portion of the third ventricle (3V), at the level of the infundibulum hypothalami (hematoxylin and eosin ×80, ×200). (**C**) Arrowhead corresponds to arrow noted in **B**, demonstrating that this tumor (T) is composed of lightly hyperchromatic, monomorphous cells with scanty cytoplasm and round nuclei with sparse chromatin. No rosette formation is seen. Serial sections demonstrate the tumor originating from the subependymal layer of the floor of the third ventricle (hematoxylin and eosin, ×400). Ep = ependymal epithelium of third ventricle; A = arcuate hypothalamic nucleus; P Pit = posterior lobe of pituitary; I Pit = intermediate lobe of pituitary; A Pit = anterior lobe of pituitary; IRe = infundibular recess of third ventricle; B = basisphenoid bone; M Re = mamillary recess of third ventricle; PitS = pituitary stalk.

from the subependymal layer of the floor of the third ventricle (Fig. 1). These cells encircle the base of the insertion of the pituitary stalk and spread anteriorly. Serial sections indicate no evidence of intracranial extension from ocular tumors.

All transgenic mice with the 1.3-kb human IRBP promoter exhibited retinal and brain tumors. As previously described,[15] sections of brain tumors revealed poorly differentiated neoplasms with histologic features found in primitive neuroectodermal tumors of infancy. Homer Wright rosettes were evident in some of the brain tumors examined.

Trilateral brain tumors appeared in early postnatal stages. Brain tumors involved mostly the diencephalon, adjacent to the floor of the third ventricle, and surrounded the habenula nuclei without involving the choroid plexus.[15] These tumors also seem to originate from the subependymal cells (Fig. 2). With extension, tumors involved the midbrain, cerebral aqueduct, and fourth ventricles.

Although lens-specific transgene expression resulted in ocular lens tumors[14] in the crystallin promoter model, brain tumors developed at a lower rate. Microscopic examination of four brain tumors from mice of the crystallin promotor model revealed that three of these neoplasms were histologically and anatomically similar to those found in BLH-promoter transgenic mice with retinoblastoma.[6] These midbrain neoplasms were located within the subependymal layer of the cerebral aqueduct and floor of the third ventricle (Fig. 3). These tumors also demonstrated cytologic features identical to those of transgenic mice with retinoblastoma.[6] These features consisted of the following: giant cells, anisonucleosis, euchromatin, hyperchromatism, indistinct cytoplasm, foci of mineralization, and a high mitotic index. The fourth mouse did not demonstrate a midbrain malignancy but harbored a choroid plexus carcinoma of the fourth ventricle, typical of that described in previous SV40 T-ag transgenic mice.[8,9]

DISCUSSION

Introduction of the SV40 T-ag oncogene into the germline of transgenic mice allows for the expression of T-ag in various tissues, often resulting in neoplasia. The site of T-ag expression may be determined by

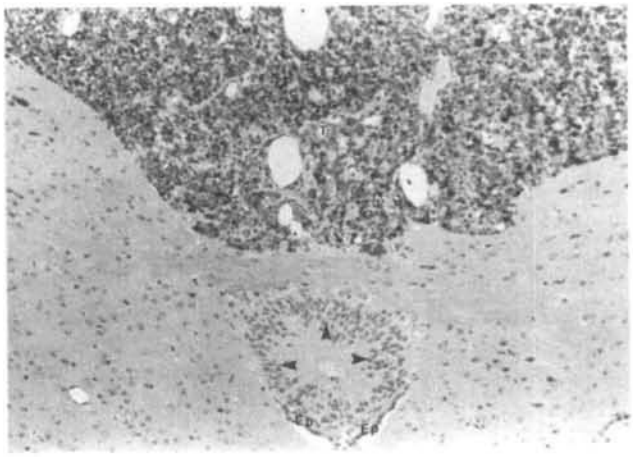

FIGURE 2. Transverse section of simian virus 40 T-antigen mouse with a 1.3-kb human interphotoreceptor retinoid-binding protein promoter demonstrating brain tumor (T) arising from the subependymal floor of the third ventricle. Dysplastic subependymal cells (*arrowheads*) are noted within the cerebral aqueduct (hematoxylin and eosin, ×100). Ep = ependymal epithelium of cerebral aqueduct; T = tumor.

the control of its natural viral enhancer (72-bp repeat region), by a tissue-specific promoter element as part of a fusion construct, by regulatory regions adjacent to or at a distance from the site of transgene integration, or by sequences residing in the SV40 T-ag gene.

The occurrence of similar central nervous system malignancies in four lines of transgenic mice containing different promoters fused to enhancerless–promoterless SV40 T-ag genes suggests the presence of some common specificity for expression of T-ag in the subependymal layer of the third ventricular floor and cerebral aqueduct in these mice. If the promoters or transgene insertion sites of these mice contained similar regulatory sequences, a common pattern of ocular tumorigenesis (and corresponding T-ag expression) would be expected. This does not occur; lens tumors, photoreceptor cell tumors, and retinoblastoma arising from the inner nuclear layer are specific to each line of mice. It is, therefore, unlikely that a common regulatory mechanism exists within the BLH promoter, alpha-crystallin promoter, human and mouse IRBP promoters, or an unidentified retinal enhancer region.

The obvious link between these transgenic mouse constructs appears to be the SV40 T-ag gene itself. Our findings suggest that a regulatory region specific to the subependymal layer of the cerebral aqueduct and floor of the third ventricle resides in the structural region of the SV40 T-ag gene.

In further support of this hypothesis, transgenic mice containing a mouse opsin promoter fused to SV40 T-ag demonstrate photoreceptor degeneration secondary to T-ag retinal expression. Interestingly, these mice also developed a PNET of the midbrain close to, or under, the ependymal cells lining the fourth ventricle.[17] The origin of these central nervous system tumors is under investigation. In addition, transgenic mice with a construct containing the human beta-actin promoter regulating human papilloma virus (HPV) 16 E6 and E7 oncogenes demonstrate similar neuroepithelial tumors associated with the ependyma of the third and fourth ventricles. Thus, it seems that a regulatory region specific to this area of the central nervous system also may reside in the structural region of the HPV E6 and E7 oncogenes.[18]

Our hypothesis is supported by the proposal of Messing et al[19] that SV40 T-ag sequences may play a role in the tissue-specific expression of T-ag to choroid plexus, thymus, and kidney. Although the 72-bp enhancer appears to be important in the development of choroid plexus papillomas and in nonmalignant lesions of the kidney and thymus,[8] thymic and choroid plexus lesions have occurred in mice lacking the SV40 enhancer.[9,20] The existence of regulatory elements

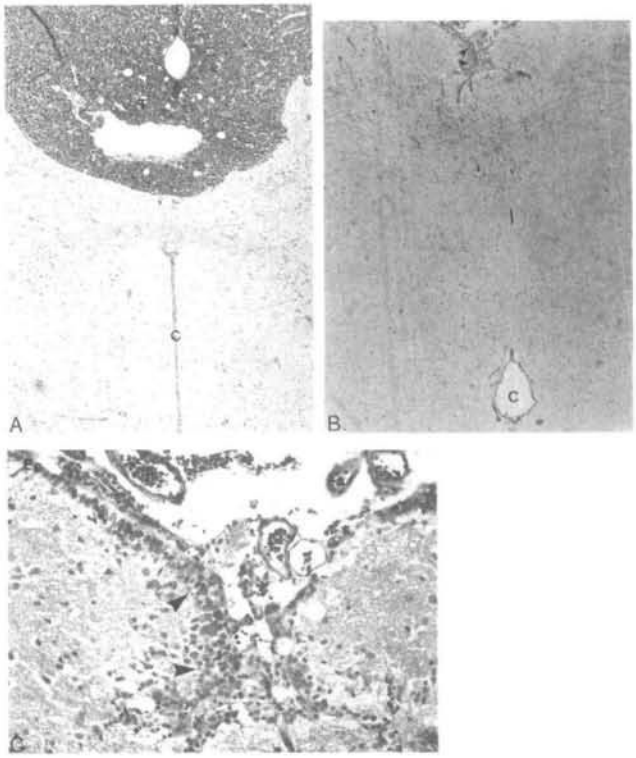

FIGURE 3. (**A**) Transverse section of a simian virus 40 T-antigen mouse with an alpha A-crystallin promoter demonstrating a brain tumor arising from the floor of the third ventricle (hematoxylin and eosin, ×100). (**B**) Low- and (**C**) high-power views from a transverse section of an early brain tumor from simian virus 40 T-antigen mouse with an alpha A-crystallin promoter identifying tumor arising from the subepehdymal layer (*arrowheads*) of the floor of the third ventricle (hematoxylin and eosin, ×40, ×400). 3V = third ventricle; C = cerebral aqueduct; Ep = ependymal epithelium of third ventricle; T = tumor.

within the SV40 T-ag gene is supported further by our finding of a choroid plexus tumor in the alpha A-crystallin mouse.

If the development of this malignancy is determined by SV40 T-ag sequences, why has this tumor not been previously identified? There are reasons for the lack of reports of midbrain tumorigenesis in other SV40 constructs. With tissue-specific promoters, emphasis is placed on the predicted site of tumor formation, and examination of the central nervous system often is omitted. In addition, many lines of transgenic mice with malignancies of vital organs (i.e., heart, kidney) die before the expected onset of midbrain PNET.

The observation of midbrain PNET in the current study is the result of systematic examination of the pineal gland and suprasellar and parasellar regions of many mice, in search of the murine trilateral tumors. Because these tumors are not grossly apparent, this region must be examined microscopically to identify them. We suspect that PNET of the midbrain and third ventricle is relatively common in transgenic mice with enhancerless SV40 constructs fused to various tissue-specific promoters. Identification and isolation of the exact regulatory region within the SV40 gene specific for subependymal cells may prove valuable.

Key Words

brain tumors, retinoblastoma, SV40 T-antigen, transgenic animals, trilateral

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