Metastasizing Neuroblastomas in Mice Transgenic for Simian Virus 40 Large T (SV40T) under the Olfactory Marker Protein Gene Promoter

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ABSTRACT

The olfactory marker protein (OMP) is a 19,000 molecular weight polypeptide originally considered a selective marker for differentiated olfactory receptor neurons. In an attempt to induce neoplastic proliferation in the olfactory cells, we made mice transgenic for the simian virus 40 large T-antigen gene linked to the OMP gene promoter. Four independent lines of transgenic mice were established. Despite a high expression of the transgene in the olfactory receptor neurons, no evidence of tumor growth was observed. Instead, starting from an age of 4 months, animals of all four lines presented with highly metastatic tumors originating in the adrenal medullas or sympathetic ganglia. The histological, ultrastructural, and immunohistochemical features of the tumors were identical to those of human infant neuroblastoma. Five independent neuroblastoma cell lines were established from tumors of different transgenic animals. All cell lines constitutively express the endogenous OMP gene. The transgene product, simian virus 40 large T-antigen, associates with the product of the anti-oncogene p53 in these cell lines. This transgene system not only offers a biologically faithful model for investigations on the pathogenesis of neuroblastoma, the most common solid neoplasia of infancy, it also raises intriguing questions about the role of the OMP gene for the differentiation of the sympathetic neurons.

INTRODUCTION

The OMP is a 19,000 molecular weight polypeptide that was originally found and purified by F. Margolis from mouse olfactory mucosa (1). The expression of OMP in the olfactory neuroepithelium is developmentally regulated. OMP is abundantly present in the cytoplasm of differentiated olfactory receptor neurons but is not detected in the neural basal precursor cells (2, 3). OMP has, therefore, been considered a specific marker for functionally mature olfactory receptor cells (4). Heteroantisera against rat OMP detect immunoreactive protein in the olfactory receptor neurons of all mammalian species studied (5, 6).

Cloning of the rat OMP gene and its cDNA has revealed a single exon gene with no significant nucleotide sequence homology to any other known gene (7, 8). The biological role of OMP has remained elusive. Based on the coinciding functional maturation of the olfactory receptor neurons and the expression of OMP, it has been hypothesized that OMP is involved in the transmission of olfactory signals (9).

Baker et al. (10) challenged the prevailing concept of a selective expression of OMP in the olfactory receptor neurons. They presented immunohistochemical evidence for low levels of OMP in discrete neurons in the cerebellum, hypothalamus, and spinal cord of rat and hamster, indicating that OMP might be a more ubiquitously distributed neural protein. The close linkage between the OMP gene and the mouse deafness mutation shaker 1 reported by Brown et al. (11) further indicates involvement of the OMP gene outside the olfactory system. Moreover, Evans et al. (12) have recently found that the human OMP gene is a strong candidate gene for the usher syndrome 1 (an autosomal recessive blindness and deafness disorder).

We recently isolated the murine OMP gene from a murine phage library. Given that OMP constitutes an exclusive marker for the olfactory receptor neurons, we attempted to induce neoplastic proliferation of these cells by making mice transgenic for the SV40T under the OMP gene promoter region. Here we report the establishment of four separate lineages of mice transgenic for the SV40T oncogene under the OMP promoter. The transgenic mice did not, however, develop tumors in their olfactory membranes, despite a high expression of the transgene in the olfactory receptor neurons. Instead, all lineages of transgenic mice presented with metastatic tumors originating in the adrenals or sympathetic ganglia. These neoplasms were morphologically, ultrastructurally, and biochemically counterparts of childhood neuroblastomas. The tumorigenesis in the OMP-SV40T transgenic mice provides a faithful model for the most common solid malignancy of human infancy. In addition, it also gives further support for the involvement of OMP in the development and differentiation of the sympathetic nervous system.

MATERIALS AND METHODS

Isolation of the Murine OMP Gene Promoter Region. The OMP gene was isolated from a murine phage library prepared of DNA from the cell line NIH3T3 (kindly provided by Mark Moore, Scripps Clinic and Research Foundation). A PvuII fragment of 270 base pairs from the rat OMP cDNA clone pOMP-3 (7) was used to isolate a phage clone. The clone contained all of the translated sequence (492 base pairs) as well as 566 base pairs of 3' untranslated region and 10 kilobases of 5' flanking region. Subclones were established, and the sequence was determined for all of the transcribed parts of the OMP and 330 base pairs of the 5' flanking region (data not shown).

Fusion Gene Construction. To join the OMP promoter to the SV40T gene, two complementary oligonucleotides of 83 nucleotides were synthesized. They cover the 5' flanking sequence of the OMP gene from position 1 to a BamHI restriction site 70 base pairs upstream of the OMP initiation codon. To facilitate the construction work further, it also contains a linker with sequences for the restriction sites, EcoRV, BglII, and Sall (Fig. 1). This oligonucleotide was inserted into the vector pBBS+ (Stratagene, La Jolla, Ca), which had been digested with the restriction enzymes BamHI and SalI. The SV40 early region, including the coding sequences of the transforming large tumor (T) and small tumor (t) antigens (13) and extending from the Stul (position 5187 in SV40 genome accession number J02400) to the BamHI (position 2533) restriction site, was excised from pBSV-3x (14). The pBS+ oligonucleotide construct was digested with EcoRV and BglII, and the SV40T...
fragment was inserted. A subclone of the OMP 5’ flanking region containing a 6-kilobase long BamHI fragment was used as the source for the remaining parts of the OMP promoter region. This BamHI fragment was cloned into the BamHI site of the above-mentioned construct. Correct orientation of this fragment was verified by restriction enzyme digestion.

**Production of Transgenic Mice.** DNA was prepared from the above-described fusion gene, and it was purified twice by CsCl equilibrium centrifugation. The fragment to be used for microinjection was excised from the plasmid by digestion with the restriction enzymes StuI and SphI. The fragment was isolated from a TAE agarose gel using glass bead extraction from a TAE agarose gel; thereafter, the fragment was purified over an Elutip column (Schleisser & Schuell). It was dissolved to a final concentration of 1 mg/ml in TAE and digested with proteinase-K (1 mg/ml) in 20 mmol buffer [1% SDS, 50 mmol Tris-HCl (pH 8), and 20 mmol NaCl] for 1—2 h. The digest was diluted 1:10 in water and boiled for 10 min to inactivate proteinase-K. Four μl were added as template to PCR with oligonucleotides (CAT AAT TCA AGC AAA GGC AGC TCG and CAG CGT GTT GAT CCT GTT TGA GC) as primers. All nucleic acid and recombinant DNA techniques were carried out according to procedures compiled by Maniatis et al. (17).

**Antibodies.** Goat and rabbit antisera against rat OMP were kindly donated by Dr. F. Margolis and a rabbit antisera made against recombinant SV40Tag by Drs. Christine Jolicoeur and Douglas Hanahan (UCSF, CA). Monoclonal antibodies against M, 68,000, 160,000, and 200,000 neurofilaments were from Boehringer-Mannheim. PAb122, a hybridoma secreting an anti-p53 monoclonal antibody, was obtained from the American Type Culture Collection.

**Morphological and Immunohistochemical Methods.** Tissue was fixed in zinc-formalin and paraffin embedded. Rehydrated 5-μm sections were stained with hematoxylin-eosin. Small fragments of fresh tumors were fixed in 2% glutaraldehyde in cacodylate buffer and routinely processed for transmission electron microscopy. Impprints from freshly cut surfaces of tumor tissue, 5-μm sections, and cells cultivated on Tissue-Tek chamber slides were fixed for 5 min in cold (−20°C) acetone, and rehydrated sections from paraffin-embedded tissue were treated with 10% normal human serum in PBS for 1 h to prevent nonspecific binding of antibodies. The primary antibodies at appropriate dilutions in PBS-normal human serum were added for 1—2 h, and after repeated washings, indicated fluorochrome-conjugate secondary antibodies were added. The slides were mounted in PBS-glycerol (pH 8).

**ELISA Assay.** ELISA plates (96 wells) were coated overnight with recombinant SV40Tag protein (kindly donated by Dr. Bruce Stillman, Cold Spring Harbor, NY) at 100 mg/ml in PBS (pH 8). Sera from mice were diluted 1:300, 1:100, and 1:1000 and added to triplicate wells. After 1 h at room temperature, the plates were washed and diluted 1:1000 in alkaline phosphatase-conjugated donkey-anti-mouse immunoglobulin. The plates were washed after 1 h at room temperature; p-nitrophenyl phosphate (Sigma Chemical Co.; 750 μg/ml, pH 8) was added, and the absorbance was quantitated in a spectrophotometer.

**Northern Analysis.** Total RNA was prepared from cell lines and tissues by acid guanidinium thiocyanate-phenol-chloroform extraction (18). Agarose gels for Northern analysis were prepared and run as described by Maniatis et al. (17) with the following modifications. Denaturing 1.5% agarose gels contained 1X 4-morpholinepropanesulfonic acid buffer, 0.6% formaldehyde, and 0.2 μg/ml ethidium bromide. The gels were equilibrated in 10X SSC (1X SSC: 0.15M NaCl, 0.015M Na2 citrate) for 30 min before capillary blotting overnight onto nylon membranes (Magna NT; Micron Separations, Inc.). The RNA was immobilized on the filter by baking at 80°C for 1 h.

Prehybridization was carried out at 42°C for 6 h in 50% deionized formamide, 5X saline-sodium phosphate-EDTA, 5X Denhardt’s solution, and 0.5 mg/ml denatured salmon DNA. Hybridization was carried out overnight at 42°C in 50% deionized formamide, 5X saline-sodium phosphate-EDTA, 1X Denhardt’s solution, 0.1 mg/ml denatured salmon sperm DNA, and 106 cpm/ml random hexamer-labeled probe. Filters were washed three times for 30 min in 2X SSC-0.1% SDS at 65°C. Autoradiography was carried out at −80°C with an intensifying screen.

**Southern Blotting.** Genomic DNA from tumors and normal tissues was prepared as described by Maniatis et al. (17). Dilutions of the genomic DNA were adjusted to a final concentration of 10X SSC, dot blotted onto filters, and immobilized by baking at 80°C for 1 h. The filters were prehybridized overnight at 65°C in 5X SSC, 4.5X Denhardt’s solution, 0.2 mg/ml denatured salmon DNA, and 0.1% SDS. To this solution was added random hexamer-labeled probe to a final concentration of 2 X 106 cpm/ml. Hybridization was carried out at 65°C for 8 h. The filters were washed three times for 30 min at 65°C in 2X SSC-0.1% SDS. Autoradiography was performed at −80°C with an intensifying screen.

**Metabolic Labeling and Immunoprecipitation.** Cell lines to be labeled were grown to confluency in 75-cm² flasks. The media was removed, and the cells were washed once with PBS. Methionine-free DMEM (2 ml) was added to each flask, and the cells were incubated at 37°C for 15 min. [35S]methionine (final concentration, 250 μCi/ml) and 20 μl 1 m 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid were then added. The cells were cultured for 3 h at 37°C, 6% CO2. The cells were then washed with Tris-buffered saline and lysed in 1% Triton X-100, 0.5 mm phenylmethylsulfonyl fluoride, 1X Tris-buffered saline for 15 min at 4°C. Nuclei and cellular debris were removed from the lysates by centrifugation at 4000 x g for 10 min. Preclarification of metabolically labeled lysates was carried out using normal mouse serum, normal rabbit serum, and Protein A-Sepharose (Pharmacia). Lysates were incubated with appropriate antibodies overnight at 4°C. Immune complexes were precipitated with Protein A-Sepharose. Immunoprecipitated proteins were analyzed on 7.5% SDS-polyacrylamide gels under reducing conditions.

**RESULTS**

**Production of Mice Transgenic for OMP Promoter-SV40T.** Eight surviving transgenic founder animals were obtained from fertilized ova of SJL x C57BL/6 F1 hybrids injected with a chimeric gene construct consisting of 6 kilobases of the OMP promoter region linked through a 83-base pair oligonucleotide to a 2.7-kilobase fragment containing the SV40 early region, including the coding sequences of the transforming large tumor (T) and small tumor (t) antigens (Fig. 1). The presence of the transgene was assessed by dot-blot hybridization of tail DNA from 4-week-old mice or by PCR with DNA isolated from single toe cuts of 1-week-old pups as template. Four lines (nos. 7, 13, 37, and 50) of transgenic mice were established. They displayed normal development and mating behavior, but some females were notoriously cannibalizing and thus poor breeders.

**Expression of SV40T in the Olfactory Mucosa.** Given that the expression of OMP is initiated in the olfactory neurons early in the last trimester of gestation (19), histological sections were made from the forehead of newborn TG+ (Fig. 2a) and control, TG− pups (Fig. 2b). Expression of the SV40T transgene apparently did not seriously impair development of the olfactory mucosa. Discrete areas with slightly disturbed cellular organization, mitotic figures in the superficial layers, and scattered leukocytes could be observed in the olfactory mucosa of TG+ mice (Fig. 2a). Immunostaining with a goat antiserum against rat OMP gave a strong cytoplasmic decoration of
the receptor neurons in the olfactory mucosa of both TG+ and TG− pups with similar frequencies of OMP-containing cells (Fig. 2, c and d). The olfactory receptor neurons of TG+ mice displayed a strong nuclear reactivity with rabbit antisera against SV40Tag (Fig. 2e). A similar abundant presence of immunoreactive Tag was seen in the nuclei of the olfactory receptor neurons of TG+ mice of all ages studied. No indication of neoplastic transformation or tumor growth was found in the noses of TG+ mice.

**Tumorigenesis in TG+ Mice.** Starting from an age of 4 months, TG+ mice presented with tumors originating in the sympathetic nervous system. The highest incidence of neoplasms was seen in mice of lineage no. 7, where 73% of the animals presented with neoplasms during a follow-up period of 10 months. The incidence of tumors in lineage no. 50 was 24%; in lineage no. 13, 18% percent; and less than 10% in lineage no. 37. The TG+ mice of lineage no. 7 also developed tumors at a younger age than did mice of the other three lineages (Fig. 3).

Most of the tumors originated in the adrenal medulla. Autopsy of mice, killed in a moribund state, typically revealed bilateral adrenal tumors sized 20 mg up to 5 g, with multiple metastatic nodules in liver, lungs, and para-aortic lymph nodes. Three animals presented with primary paraspinal tumors in the thoracic cavity, apparently originating in the sympathetic trunk. One mouse presented with a tumor in the neck, probably originating in the sympathetic cervical ganglia; one developed paraplegia caused by an intra-abdominal paraspinal tumor infiltrating the spinal cord; and one animal was moribund from an acute urinary retention due to a presacral tumor with obstructive infiltration into the bladder neck. Several TG+ mice were found dead with intraabdominal bleeding from spontaneously ruptured, highly vascularized adrenal tumors. Autopsy of more than 20 TG+ newborn pups and histological examination of their adrenals frequently revealed small clusters of hyperchromatic, undifferentiated cells in the medullas, but overt tumors were not recorded.

**The Tumors Induced by OMP-SV40T Are Phenotypically Neuroblastosomas.** The tumors originating in the adrenal medullas and sympathetic trunk were histologically composed of small, uniform, basophilic round or ovoid cells with a scanty cytoplasm (Fig. 4a). Occasionally, larger ganglioid cells could be seen. Mitotic figures were frequent. The tumor cells showed a tendency towards grouping into Homer-Wright rosettes and palisades (Fig. 4b). Some tumors contained more differentiated areas with decreased cellularity and a

![Fig. 2. Histology of hematoxylin & eosin-stained sections of the olfactory mucosa from newborn (a) TG+ and (b) TG− mice. Immunostaining of olfactory receptor neurons with goat antiserum against rat-OMP in sections of olfactory mucosa from (c) TG− and (d) TG+ mice. The strong nuclear expression of the transgene product, SV40Tag, in the olfactory mucosa of TG+ mice is demonstrated in (e) by immunostaining with rabbit antibodies to Tag.](image-url)
The tumors were mostly highly vascularized, but many larger tumors still contained necrotic areas with viable cells grouped around the vessels.

Examination by transmission electron microscopy revealed typical dense-core neurosecretory cytoplasmic granules in the tumor cells (Fig. 4c). The cellular morphology in the metastatic lesions was identical to that of the primary tumors (Fig. 4, d-f). The histological and ultrastructural picture was identical to that of human infant neuroblastoma. Immunohistochemical staining of imprints and sections from the tumors disclosed expression of SV40T in the nuclei of the tumor cells (Fig. 4g). A weak but reproducible intracytoplasmic reactivity with anti-OMP antiserum was seen in the tumors (Fig. 4h). A consistent finding was a very low or undetectable surface expression of major histocompatibility antigen (H-2) class I molecules on the tumor cells. Fluorescence microscopy of tumor imprints, frozen sections, or FACS analysis of freshly isolated tumor cells immunostained with a rat MoAb 1/42 against mouse common class I MHC antigens gave virtually negative results. Only occasional tumor cells in imprints or frozen sections displayed reactivity appreciable over the background (data not shown).

The N-myc Gene Is Not Amplified in the Primary Tumors. Amplification of the cellular protooncogene N-myc is frequently found in tumors of advanced stages of childhood neuroblastoma (20, 21). Genomic DNA was extracted from tumor tissue of four TG+ mice and hybridized at serial dilutions in a dot-blot assay with a 32P-radiolabeled mouse N-myc cDNA probe. The signals obtained from the tumor DNA did not, however, differ from those from the corresponding genomic tail DNA preparations (Fig. 5). This indicates that amplification of N-myc is not a crucial initial event for generation of neuroblastomas in TG+ mice.

TG+ Mice Are Not Immunologically Tolerant to SV40T. SV40-transformed cells are highly antigenic in several mouse strains (22, 23), and tissue from mice expressing SV40T as a transgene provokes graft rejection responses in the syngeneic parental strains (24). Serum was collected from TG+ mice and analyzed for the presence of Tag antibodies in an ELISA assay. Four-week-old TG+ mice did not have measurable levels of antibodies against Tag, but by the age of 5 months, most of the TG+ mice showed a humoral immune response against the transgene product (Fig. 6). This apparent lack of immunological tolerance to Tag suggests that the transgene is expressed rather late during ontogeny and/or at locations not in intimate contact with the developing immune system. Although scattered leukocytes were seen in the olfactory membrane of TG+ mice, the main site of immunization could still be the adrenals. Histological examination of adrenals from TG+ mice, with no evidence of tumors, revealed lymphocytic infiltrations, signs of medullary cell destruction, and hemorrhagic areas at the cortico-medullary border (Fig. 7). We did not, however, find evidence for a protective effect of the serum antibodies against occurrence of neuroblastomas. Significant titers of antibodies to Tag were also found in sera of tumor-bearing TG+ mice.
NEUROBLASTOMAS IN OMP-SV40T TRANSGENIC MICE

No specific reactivity was obtained with anti-cytokeratin antibodies (not shown). An intracytoplasmic reactivity with a rabbit antiserum against rat neuron-specific enolase and with a monoclonal antibody against synaptophysin was also seen in all cell lines (data not shown). Immunostaining with a rabbit antiserum against SV40 Tag displayed a nuclear accumulation of the transgene product in all cell lines (Fig. 8c). Tag is known to interact with several intracellular proteins, including the products of the recessive oncoproteins retinoblastoma Rb-i (25) and p53 (26, 27). Nuclear colocalization of Tag and p53 was demonstrated by double immunostaining with a monoclonal antibody against p53 and rabbit anti-Tag (Fig. 8, c and d).

To further demonstrate the physical interaction between Tag and p53, detergent lysates from [35S]methionine-labeled cells were immunoprecipitated with anti-Tag antibodies and with anti-p53 antibodies. Autoradiographs of the precipitates, separated on SDS-polyacrylamide gel electrophoresis, revealed coprecipitation of p53 and Tag (Fig. 9). Only a weakly labeled band was obtained with anti-Tag and anti-p53 in the Mr 107,000—110,000 region corresponding to the expected migration of the Rb-i gene product.

A weak cytoplasmic reactivity was seen in all cell lines with goat-anti-OMP antibodies (Fig. 8a). To confirm the SV40T and OMP expression in the cell lines, total RNA was isolated from each line and analyzed by Northern blot hybridization using cDNA probes to SV40 early gene regions and mouse OMP. Two species of SV40 message of 2.6 and 3.1 kilobases were detected in all neuroblastoma lines and also in the positive control cell line, cos 7 (Fig. 10A). The OMP cDNA probe hybridized to a 2.6-kilobase message in the neuroblastoma cell lines (Fig. 10B), indicating a constitutive transcription of the endogenous OMP gene. Probing Northern blots with the mouse N-myc cDNA gave an enhanced signal in two of the cell lines (7.69 and 13.6), indicating amplified N-myc expression (Fig. 10C).

DISCUSSION

Studies on tumorigenesis in transgenic animal models have disclosed important information about the functions of oncoproteins in...
Fig. 8. Cell line 7.45 cultivated on a slide and immunostained with goat antibodies to rat OMP (a) and with monoclonal antibody to M, 66,000 neurofilaments (b). The nuclear colocalization of p53 and SV40Tαg demonstrated by double immunostaining of the same culture with monoclonal antibody FAB122 to p53 (c) and rabbit anti-Tag (d).

**In vivo.** By introducing the oncogenes under control of regulatory elements of genes with their physiological expression restricted to certain cells or tissues or to defined stages of development, anatomical and/or temporal targeting of oncogenesis has been achieved (reviewed in Refs. 28 and 29).

Here we describe a biologically faithful transgenic mouse model for neuroblastoma, the most frequent solid neoplasia of human infancy. While many of the tumors obtained in transgenic animals have no or only a moderate metastatic potential, the neuroblastomas of the OMP-SV40 transgenic mice described here more closely resemble their human counterparts in regards to their strong metastatic propensity.

The SV40T antigen gene is a potent oncogene which has the capacity to immortalize and transform various cells both *in vitro* and *in vivo*. The Tag coding sequence has, therefore, been frequently used in chimeric gene constructs to induce tumors in transgenic mice (30–33). SV40T represents a real, exogenous viral oncogene since no corresponding cellular protooncogene has been identified. Still, an intimate interplay between the oncogene product, Tag, and cellular growth regulatory proteins is pivotal for transformation and immortalization to take place. Among these are p53, originally discovered due to its physical association with Tag (26, 27). Also another recessive oncogene product, p105Rb, associates with Tag in SV40-induced tumors (34).

Windle *et al.* (35) reported the occurrence of retinoblastomas in mice transgenic for SV40T under the promoter of the lutenizing hormone β-subunit gene. They found evidence for an interaction between Tag and p105Rb in the induced retinoblastomas. In the cell lines derived from the neuroblastomas described here, Tag appears mainly to coprecipitate with p53, while p105Rb seems to be expressed at relatively low levels. This finding is not unexpected since there are previous reports indicating high levels of p53 expression in spontaneous mouse neuroblastomas lacking Tag (36).

The selective targeting of the tumorigenesis by SV40T under the OMP promoter gene to the sympathetic nervous system was surprising in light of existing knowledge. Aguzzi *et al.* (37) recently reported a similar distribution of sympathetic neuroblastomas in a single line of mice transgenic for a cDNA to polyoma virus middle-T antigen linked to the thymidine kinase promoter. Besides the neuroblasta line,
they described three transgenic animals, one without any detectable phenotype and two that died with hemangiomas. As the thymidine kinase gene is physiologically expressed in a variety of tissues, they concluded that a specific integration site of the transgene construct might account for the selectivity of tumorigenesis. Although the site(s) of integration of the transgene construct reported here are still to be identified, the identical distribution patterns of oncogenesis in four different lineages of transgenic mice strongly indicates a decisive role for the activity of the OMP promoter region. A further indication of the impact of the OMP promoter region on the tumor targeting is the constitutive expression of the endogenous OMP gene in all of the five tumor cell lines we established.

Recently, Largent et al. (38) reported a single mouse line transgenic for SV40T under the control of 3 kilobases of the rat OMP promoter region. These transgenic animals presented with hypoplastic olfactory mucosa and developed olfactory tumors, originating in the nasal cavity, at a low frequency (3% of the hemizygous and 7% of the homozygous by 1 year). The much higher incidence of tumors and their different distributions in our transgenic lines might be due to the additional regulatory elements included in the larger (6-kilobase) mouse OMP promoter region used.

Taken together, these findings suggest that the expression of the OMP gene is not restricted to the olfactory receptor neurons or to selected cells in the central nervous system. It is tempting to speculate that OMP is involved also in the development of the sympathetic nervous system. OMP might not be functional in the embryonal neural proliferation but may be functional during discrete stages of terminal neural differentiation. The neuroblastoma cell lines we report here, all of which constitutively express the OMP gene, provide an additional tool for investigations on the molecular functions of OMP.

It is intriguing that we have not found neoplastic proliferation in the olfactory neuroepithelium of the TG+ mice, despite a high level of continuous Tag expression. The olfactory neurons are unique neural cells since they retain their proliferative capacity throughout life and are continually renewed. This steady proliferating tissue might be endowed with an unusually stringent internal control of differentiation to protect against malignant transformation. Given that OMP is a marker of mature olfactory receptor neurons, the concomitant olfactory expression of Tag in TG+ animals might be restricted to cells that have terminally exited their proliferative cycle and are not amenable to transformation. It is also possible that potentially neoplastic cells are shed off before the establishment of a tumor. A similar mechanism has been suggested to explain the surprisingly low incidence of epithelial tumors in the intestinal mucosa. It should, however, be kept in mind that the olfactory neurons are not entirely resistant to tumorigenesis. The rare cases of neuroblastoma in human adults, the esthesio-neuroblastoma, originates in the olfactory membrane. Moreover, Koike et al. (39) reported the occurrence of olfactory neuroblastomas in mice transgenic for the early region genes E1A and E1B of human adenovirus 12 under the mouse mammary virus long terminal repeat promoter.

Faas et al. (40) reported a major impact of the ability to mount an immune response against the transgene product on the incidence of choroid plexus tumors in mice transgenic for the SV40 Tag genes as originally described by Brinster et al. (41). Transgenic animals of a subline neonatally tolerized to SV40Tag presented with a high frequency of tumors, while immune responsiveness conferred virtual resistance to SV40Tag-induced oncogenesis. The role of the immune response in the development of the neuroblastomas described here remains to be elucidated. Most, if not all, of the OMP-SV40T transgenic mice developed a humoral immune response against SV40Tag, and antibodies were also present in the sera of tumor-bearing animals. The presence of active cell-mediated anti-Tag responses has not been assayed in the TG+ mice, but the tumor lesions did not show morphological signs of immune rejection. Accumulation of inflammatory cells was, on the other hand, frequently observed in the adrenal medullas of young TG+ mice without tumors. Together with the observed absence or very low surface expression of MHC class I antigens on the neuroblastoma cells, it is tempting to speculate that selected SV40T transformed cells with no or very low class I antigen expression escape the immune surveillance and form tumors. It should, however, be appreciated that neuronal cells constitutively display a very low membrane expression of major histocompatibility complex molecules. To address the question of a possible impact of the immune responsiveness on the incidence of neuroblastomas in the OMP-SV40 transgenic model, the transgene is now being bred into congenic strains of mice known to be immunologically high-versus low-responders to SV40Tag.

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REFERENCES


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