A Simian Virus 40 Large T-Antigen Segment Containing Amino Acids 1 to 127 and Expressed under the Control of the Rat Elastase-1 Promoter Produces Pancreatic Acinar Carcinomas in Transgenic Mice

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Received 11 April 1997/Accepted 23 July 1997

The simian virus 40 large T antigen induces tumors in a wide variety of tissues in transgenic mice, the precise tissues depending on the tissue specificity of the upstream region controlling T-antigen expression. Expression of mutant T antigens that contain a subset of the protein's activities restricts the spectrum of tumors induced. Others showed previously that expression of a mutant large T antigen containing the N-terminal 121 amino acids (T1-121) under control of the lymphotropic papovavirus promoter resulted in slow-growing choroid plexus tumors, whereas full-length T antigen under the same promoter induced rapidly growing CPR tumors, T-cell lymphomas, and B-cell lymphomas. In those instances, the alteration in tumor induction or progression correlated with inability of the mutant large T antigen to bind the tumor suppressor p53. In the study reported here, we investigated the capacity of an N-terminal T antigen segment (T1-127) expressed in conjunction with small t antigen under control of the rat elastase-1 (E1) promoter to induce pancreatic tumors. The results show that pancreases of transgenic mice expressing T1-127 and small t antigen display acinar cell dysplasia at birth that progresses to neoplasia. The average age to death in these mice is within the range reported for transgenic mice expressing full-length T antigen under control of the E1 promoter. These results indicate that sequestering p53 by binding is not required for the development of rapidly growing acinar cell carcinomas. In addition, we provide evidence that small t antigen is unlikely to be required. Finally, we show that the p53 protein in acinar cell carcinomas is wild type in conformation.

The simian virus 40 (SV40) early gene region encodes two major proteins, the large T (94-kDa [94K]) and small t (18K) antigens, each of which participates in the transformation of cells in culture (for recent reviews, see references 10 and 22) and the development of tumors in immunocompromised (49) and transgenic (1, 11, 14) animals. The roles of the large T and small t antigens in transformation of cells in culture and in tumorigenesis have been investigated intensely. Large T antigen is sufficient to fully transform cells and to induce tumors in nude and transgenic mice. In rodent cells, small t antigen, in general, acts as an auxiliary transforming protein that assists in transforming cells in vitro when large T antigen is in limited amounts (3), when cells are quiescent (31, 49), or in cells that are minimally transformed by large T antigen (52) or that require small t antigen for metabolic stabilization of p53 (53, 44); in transgenic mice, small t antigen participates in inducing tumors in slowly dividing epithelial cells (8).

Large T antigen alone or in combination with small t antigen induces tumors in a wide variety of cell types in transgenic mice. The specific cell types involved depend on the tissue-specific promoter controlling T-antigen synthesis (1, 11, 14). Investigations of mutant T antigens have pinpointed the involvement of specific T-antigen activities in the generation and progression of specific types of tumors. For instance, the capacity of large T antigen to bind the tumor suppressor protein p53 influences both the types of tumors that develop and their kinetics of appearance. Full-length large T antigen expressed

under control of the lymphotropic papovavirus (LPV) transcriptional control region induces tumors in choroid plexus epithelium (CPE), T lymphocytes, and B lymphocytes in transgenic mice (6). In contrast, an N-terminal T-antigen segment containing 121 amino acids (T1-121) induced CPE tumors only (6, 33). In addition, the CPE tumors grew more slowly than were their counterparts expressing full-length T antigen (33, 41). When T1-121-transgenic mice were bred with p53-null mice, rapidly growing CPE tumors developed, and tumors that developed in T1-121/p53-heterozygous mice displayed loss of heterozygosity at the p53 locus (41). In addition, T1-121/p53-null mice developed T- and B-cell lymphomas. Similarly, inactivation of p53 by coexpression of T1-121 and a p53 carboxy-terminal dominant-negative fragment resulted in aggressive CPE tumors (4). Those results indicated that the spectrum of tumors induced by mutant T antigens depends on specific activities that they retain and emphasized the importance of inactivating p53 in the development of aggressive CPE tumors and lymphomas. Whether removing the p53-binding capacity from T antigen would alter the induction or progression of tumors in other tissues is not clear.

Full-length T antigen's capacity to induce acinar cell carcinomas in transgenic mice has been investigated in detail (13, 21, 26, 27). The studies reported herein were designed to determine whether an N-terminal T-antigen fragment was sufficient to induce pancreatic acinar tumors or, alternatively, activities from additional T-antigen regions would be implicated in tumorigenesis. We show that a transgene producing an N-terminal T-antigen segment containing amino acids 1 to 127 (T1-127) and small t antigen is sufficient to induce acinar cell

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FIG. 1. Survival of T1-127-transgenic mice. The T1-127 founder and mice of his lineage were euthanized when they were clearly in poor health or had distended abdomens. The percentage of animals that showed no sign of illness at each time point is plotted versus age in weeks.

dysplasia, carcinomas, and metastases. In addition, we show that the p53 in the tumors is phenotypically wild type.

MATERIALS AND METHODS

Cell lines. The cell line B6/pPVU0, described previously (19), expresses wild-type large T and small t antigens. The cell line B6/C-3 was derived from C57BL/6 mouse embryo fibroblasts exposed to UV-irradiated human cytomegalovirus; it expresses mutant p53 (45).

Cloning N-terminal large T-antigen coding sequences 3' to the rat E1 promoter. A mutant T-antigen gene containing the coding sequences for the first 127 amino acids of large T antigen and small t antigen was cloned 3' of the 4.5-kb rat elastase-1 (E1) gene upstream control region described by Quaife et al. (30). This upstream region results in high-level expression which is limited to pancreatic acinar cells in transgenic mice (26, 30, 40).

More specifically, the 4.5-kb E1 upstream region was released from plasmid pRE/p53G-ala135 (a gift of A. Levine) by *Cla*I and *Xho*I digestion and cloned between the corresponding sites of the vector pGC2 (24) to produce plasmid pGC2-E1, to which a polyadenylation signal then was added. Accordingly, plasmid WT-2, which contains the SV40 early region cloned at the *Bam*HI site of pBR322, was cleaved with *Bcl*I, the resulting extension was filled in with Klenow polymerase, and a *Bgl*II linker then was added. A 237-nucleotide fragment released by *Bgl*II-*Bam*HI digestion was inserted at the *Bam*HI site of plasmid pGC2-E1. The orientation of the insert was determined by *Hpa*I digestion. Next, the *Xho*I site of plasmid pGC2-E1-polyA was converted to a blunt end, and an *Mlu*I linker was added. Finally, the *Bgl*II site of an SV40 DNA containing a 34-bp deletion (nucleotides 4434 to 4401) was converted to a blunt end, and an *Mlu*I linker was added. From the resulting SV40 DNA, the *Mlu*I-*Bam*HI digestion fragment, containing SV40 nucleotides 5235 to 2533, was inserted between the *Mlu*I and *Bgl*II sites of plasmid pGC2-E1-polyA. The resulting plasmid (pE1-T1-127) is expected to express an N-terminal fragment of T antigen, containing the first 127 amino acids followed by four amino acids (Asn, Gln, Asn, and Cys) from the shifted reading frame, under the control of the rat E1 promoter region.

To prepare pE1-T1-127 for microinjection, the construct was digested with *Cla*I and *Bgl*II, and the segment containing the E1 control region, large T- and small t-antigen coding segment, and poly(A) signal was recovered from an agarose gel following electrophoresis and purified by the GeneClean procedure (20). The DNA was eluted in injection buffer (10 mM Tris, 0.1 mM EDTA [pH 7.4]), passed through a 0.2- μ m-pore-size filter, and diluted to 4.5 μ g per ml.

A construct containing the coding sequence for the first 147 amino acids of large T antigen also was cloned 3' to the 4.5-Kb E1 upstream region. The source of the mutant T-antigen coding sequence was plasmid pT147NS (38) (kindly provided by L. Sompayrac). This plasmid contains an SV40 early region in which a stop codon replaces codon 148 in the vector pcDL-SR α (38). The SV40 sequences between nucleotides 4568 and 5135 had been replaced with the corresponding segment of a cDNA copy of large T-antigen mRNA. Therefore, the construct produces a truncated large T antigen containing amino acids 1 to 147. No small t antigen is produced. A DNA segment containing the splice site, T-antigen coding region, and poly(A) site was released from pT147NS by digestion with *Xho*I and *Eco*RI. The released segment was inserted into the vector pGC2E1 between the corresponding sites to produce plasmid pE1-T147.

Generation of transgenic mice. Fertilized mouse embryos at the one-cell stage were recovered from superovulated female B6D2F1/J mice that had been mated 20 h earlier to C57BL/6 males. The solution of the purified DNA fragment was injected into a pronucleus of each embryo. The microinjected embryos were implanted through the infundibulum of pseudopregnant female mice. Superovu-

lation, microinjections, and implantations were performed as described elsewhere (16).

Pups were weaned at 3 to 4 weeks of age; DNAs extracted from sections of the tails were screened for the presence of the transgene by PCR analysis by use of three sets of T-antigen primer pairs: 5'-4925 to 4945-3'-3'-5118 to 5139-5', 5'-4055 to 4072-3'-3'-4271 to 4292-5', and 5'-3233 to 3254-3'-5'-3478 to 3502-3'. Amplification of the corresponding DNA 215-, 240-, and 269-bp segments indicated the presence of the transgene. Amplification of a 450-bp segment of the p53 gene by use of primers 5'-ACCGCGTGGTGGTACCTTAT-3' and 5'-TATACTCAGAGCCGGCTT-3' served as a control for integrity of the DNA. Additional controls included a PCR in which DNA was omitted, DNA from a known positive T-antigen-transgenic mouse, and DNA from a p53-null mouse. The presence of the 34-bp deletion in the T1-127 DNA was monitored by using primers 5'-4055 to 4072-3' and 3'-4546 to 4568-5', which amplify a 513-bp fragment from wild-type DNA and a 479-bp fragment from DNA containing the deletion. The transgenic line containing the T1-127 coding sequence was designated line 79. Subsequent generations were bred to C57BL/6 mice.

A purified DNA segment containing the 4.5-kb E1 transcription control region and the T1-147 coding segment also was microinjected into mouse embryos, and a T1-147-transgenic line (line 289) was derived.

Immunoprecipitation and immunoblot analyses. Extractions of proteins from cells and immunoprecipitations, using the amounts of protein extract indicated in the figure legends, were performed exactly as described in detail previously (20). Monoclonal antibodies used to immunoprecipitate large T antigen included

FIG. 2. Photographs of normal control mouse pancreas and of a pancreatic acinar cell carcinoma and liver of a T1-127-transgenic mouse. (A) Pancreatic acinar cell carcinoma removed from a female, 6.5-month-old T1-127-transgenic mouse. Note the stomach (s) and the large solid tumor attached to and almost surrounded by duodenum (arrowheads). Adjacent nonneoplastic pancreas was firmer than normal and contained multiple foci of small hemorrhagic cysts (arrows). (B) Normal control mouse pancreas (arrowhead). The spleen (arrow), stomach (s), and duodenum (d) are visible also. (C) Liver from the mouse in panel A. Note the multiple raised white foci of metastatic tumor within the liver.

TABLE 1. Histological examination of T1-127-transgenic mice

Animal no.	Transgene	Age (wk)	Moribund	Sex	Acinar cell dysplasia	Microadenomas	Acinar carcinomas	Metastases
79	T1-127, t	18	Yes	Male			+	Hepatic, mesenteric, diaphragm
79-16	T1-127, t	10	No	Female	+	+	+	None
79-17	T1-127, t	10	No	Female	+	+	+	None
79-73	T1-127, t	16	Yes	Male	+		+	None
79-9	T1-127, t	20	Yes	Female	+		+	None
79-10	T1-127, t	20	Yes	Female	+	+		None
79-4	T1-127, t	24	Yes	Female	+	+		None
79-7	T1-127, t	24	Yes	Female	+	+	+	None
79-5	T1-127, t	26	Yes	Female	+		+	Hepatic, mesenteric
289-4	T1-147	20	Yes	Female	+		+	Hepatic
289-2	T1-147	20	Yes	Male	+		+	None

PAb901, PAb419, and PAb416. These antibodies recognize epitopes contained between amino acids 684 and 698 (47), 1 and 82 (15), and 1 and 127, respectively (34) (see Fig. 5). PAb419 immunoprecipitates both large T and small t antigen. Antibodies used to immunoprecipitate p53 included PAb246 (50), PAb240 (12), and PAb421 (15). These antibodies immunoprecipitate mouse wild-type p53, mouse mutant p53, and both wild-type and mutant p53. The monoclonal antibody gD, which recognizes an epitope in the herpes simplex virus glycoprotein D (17, 18), was used as a negative control antibody. Unless stated otherwise, immunoprecipitated proteins were separated by sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis (SDS-PAGE).

For immunoblot analysis, proteins were transferred after electrophoresis to polyvinylidene difluoride (PVDF) membranes and probed as described previously (20), with the following exceptions. After incubation of blots with anti-T-antigen monoclonal antibodies, horseradish peroxidase-conjugated protein A (Amersham) was used in conjunction with the Amersham ECL (enhanced chemiluminescence) system to detect the proteins. For chemiluminescent detection of p53, blots were probed with sheep polyclonal anti-p53 antibody (Calbiochem) and detected by using anti-goat/sheep horseradish peroxidase-conjugated antibody (Sigma). This combination avoids detection of an immunoglobulin G band that under the electrophoresis conditions used might obscure the p53 band.

For extraction of proteins from tumors, tumors which had been flash-frozen in liquid nitrogen were thawed and placed in a tissue homogenizer along with 1 ml of 1% Nonidet P-40 lysis buffer (50 mM Tris [pH 8], 5 mM EDTA, 150 mM NaCl, 1% Nonidet P-40) supplemented with 1 mM phenylmethylsulfonyl fluoride, 0.01 mg of aprotinin per ml, 0.505 µg of leupeptin per ml, and 0.781 µg of pepstatin A per ml. The tissue was homogenized on ice and sonicated. Cellular debris was removed by centrifuging the lysates twice at $1,732 \times g$ at 4°C.

Necropsy and histopathology. Unless stated otherwise, the animals were euthanized by inhalation of carbon dioxide gas when they appeared to be in poor health or showed clear signs of abdominal distention. A complete necropsy was performed immediately after euthanasia, and all organs were carefully dissected and examined. Gross lesions were recorded and photographed. All organs and tissues were collected and fixed in 10% neutral buffered formalin. Bone structures were briefly decalcified in a mild buffered acid solution, and the following tissues were embedded in paraplast embedding medium, sectioned at 5 µm, stained with hematoxylin and eosin (H&E), coverslipped, and examined histologically: colon, cecum, ileum, jejunum, duodenum, stomach, pancreas, spleen, liver, gallbladder, kidney, adrenals, urinary bladder, testicles or ovaries, mammary gland, male accessory sex glands, uterus, vagina, skeletal muscle, salivary glands, lymph nodes, trachea, thyroid and parathyroid, heart, lung, thymus, brain, pituitary gland, bone, bone marrow, middle and inner ear, eyes, hardierian glands, skin, and any other tissue which contained a suspected lesion.

RESULTS

Induction of tumors by T1-127. A founder male mouse (mouse 79) was bred with C57BL/6 female mice, and the pups were screened for transmission of the transgene. At 18 weeks of age, the founder became moribund and was sacrificed; on necropsy, a pancreatic tumor was found. Thirty-four transgenic pups from his lineage were observed for 31 weeks. Thirty of these mice became moribund or showed clear evidence of abdominal distention and were euthanized. Each mouse had single or multiple pancreatic tumors. As shown in Fig. 1, approximately half of the animals developed life-threatening tu-

mors by 21 to 22 weeks of age. Complete necropsies were performed on the founder and six moribund progeny. In each mouse, one large or multiple smaller tumors were found within or replacing the pancreas. On section, the tumors were soft and gray with multiple red cystic or hemorrhagic foci. The larger tumors contained foci of necrosis and had almost completely replaced the pancreas (Fig. 2A). Three of the four largest pancreatic tumors metastasized to the liver (Fig. 2C), two invaded mesenteric lymph nodes, and one metastasized to the serosa of the diaphragm. The smaller tumors from the remaining group of animals were found within the head, body, and tail of the pancreas. Pancreas between the tumors was dense, firm, and whiter than normal. Figure 2B shows the pancreas of a normal control mouse for comparison.

Histologic examination (Table 1) of the entire pancreas of each animal revealed an absence of normal acinar tissue. Instead, the pancreases consisted of acinar cell carcinoma (Fig. 3A), acinar cell microadenomas (Fig. 3B), and dysplastic acinar cells (Fig. 3C). Occasional pancreatic islets were visible within the dysplastic acinar tissue (Fig. 3C). Metastatic carcinoma cells within the liver (Fig. 3D) were similar in appearance to the primary neoplasm. Figure 3E shows the histological appearance of normal adult mouse pancreas for comparison. No metastatic lesions were identified within the tissues other than those described above. In addition, pancreases of apparently healthy transgenic mice were examined at 10 and 16 weeks of age. Pancreatic acinar cell carcinomas were evident as early as 10 weeks after birth (Table 1). Mice bearing the T1-147 transgene also became moribund and developed pancreatic acinar cell tumors. Although fewer mice of that lineage have been investigated, all of seven transgenic mice examined developed pancreatic tumors between 20.5 and 30.5 weeks of age. As shown in Table 1, the tumors were indistinguishable in histological features from E1–T1-127 tumors.

The elastase promoter is expressed during embryonic development, with peak expression between 14 and 16 days (26, 30). As early as day 17 of fetal development, pancreatic acinar tissue of fetuses carrying a transgene that expresses full-length large T antigen and small t antigen has been shown to be hyperplastic with increased density and mitotic figures (26). We therefore examined the pancreases of T1-127 mice at birth for evidence of transgene-related pathology. All mice in a litter of seven were euthanized at 4 h after birth. Their pancreases were removed, blind coded, and prepared for histological examination. A portion of the hind limb of each pup also was removed and examined for presence of the transgene by PCR analysis. After the histological examinations were completed,

FIG. 3. Histology of acinar cell carcinoma, microadenoma, dysplasia, and metastatic acinar cells within the liver and of control adult mouse pancreas. Each panel shows a photomicrograph of an H&E-stained section at a magnification of $\times 120$. (A) Acinar cell carcinoma. Note the formation of well-differentiated acinar cell structure (a), cystic area (c), and the lack of lobule and islet formations. (B) Acinar cell microadenoma (m). The microadenoma is separated from the adjacent sheet of diffuse dysplastic acinar cells by a thin fibrous capsule and space. Note the mitotic figure (arrowhead) and apoptotic fragments (arrow) within the tissue. (C) Pancreatic acinar cell dysplasia. Note the pancreatic islet, lobular appearance of dysplastic acinar cells, mitotic figure (arrowhead), and apoptotic bodies (arrow). (D) Metastatic pancreatic acinar cells within liver (L). Note the nonencapsulated mass of acinar tissue that contains mitoses (arrowhead) and apoptotic fragments (arrow). (E) Control adult mouse pancreas. Note the pancreatic islet and adjacent normal exocrine tissue.

FIG. 3—Continued.

the correlation between the presence or absence of the transgene was revealed. Only normal pancreas was observed in each of the three nontransgenic littermates. In contrast, normal pancreas was absent from all four of the transgenic animals at birth. Only dysplastic acinar cell tissue was observed. Figure 4 shows portions of pancreas from a normal (Fig. 4B) and a transgenic (Fig. 4A) littermate.

These results indicate that expression of an N-terminal T-antigen segment (T1-121) and small t antigen is sufficient to induce dysplasia in the embryonic pancreas that progressed after birth to acinar cell carcinomas capable of metastasizing.

Expression of T1-127 and small t antigen in acinar cell tumors. Pancreatic tumors were removed from mice at autopsy and flash-frozen in liquid nitrogen. The age of the mice from which tumors were taken ranged from 18 to 31 weeks. The tumors were between 1.2 and 3 cm in diameter. Tumors from eight mice were examined for the presence of the T1-127 large

T-antigen segment and for small t antigen. As a control, proteins were extracted from a cell line (B6/pPVU0), expressing both full-length large T antigen and small t antigen. Proteins were immunoprecipitated from the extracts by the irrelevant monoclonal antibody gD, which recognizes an epitope in the herpes simplex virus glycoprotein D (17, 18), PAb901, which recognizes a C-terminal T-antigen epitope (amino acids 684 to 698) (47), PAb416, which recognizes an epitope between T-antigen amino acids 82 and 127 (reference 34 and Fig. 5), or PAb419, which recognizes an epitope in the T/t common region (15). The immunoprecipitated proteins were separated by SDS-PAGE and electrotransferred to a PVDF membrane, and T antigens were detected with PAb419. Representative results appear in Fig. 5. As expected, a band in the position expected for full-length T antigen was present in lanes to which were added proteins that had been extracted from B6/pPVU0 cells and immunoprecipitated by PAb901, PAb419, or PAb416 (Fig. 5). A band in the position expected for small t antigen ap-

FIG. 4. Histology of newborn and T1-127 pancreases. Each panel shows a photomicrograph of an H&E-stained section at a magnification of $\times 120$. (A) Pancreas of a newborn (4 h old) T1-127-transgenic mouse. Note the pancreatic islet (i), the acinar cells with increased nuclear-to-cytoplasmic ratio, and the abnormal development of lumen within acini (L) as well as numerous mitotic figures (arrowheads) and apoptotic bodies (arrow). (B) Pancreas of a newborn (4 h old) nontransgenic control mouse. Note the islet, small size of the acinar cell nucleus, the abundance of the cytoplasm, and the lack of lumen within acini.

peared only in the lane receiving the PAb419 immunoprecipitate.

Proteins immunoprecipitated from pancreatic tumors (Fig. 5) were consistent with expression of small t antigen and a large T antigen containing amino acids 1 to 127. No proteins were specifically immunoprecipitated with either the irrelevant antibody, gD, or PAb901. PAb419 immunoprecipitated a protein that migrated in the position of small t antigen and a slower-migrating band. The slower-migrating band (T1-127) was immunoprecipitated from pancreatic tumors only by PAb416. Although the T1-127 protein contains fewer amino acids than small t antigen, its slower migration most likely reflects phosphorylation of the large T-antigen N-terminal cluster of phosphorylated amino acids. In no case did a full-

length T-antigen band appeared in lanes containing immunoprecipitates of tumor cell extracts. Identical results were observed for each of the additional tumors examined (data not shown). These results show that the expected proteins were present in the tumors.

Phenotype of p53 in acinar cell carcinomas. To determine the phenotype of p53 in the tumors, proteins in extracts of the same tumors that were analyzed for T-antigen expression were immunoprecipitated with gD, PAb246, which specifically immunoprecipitates wild-type p53, PAb240, which specifically immunoprecipitates mutant mammalian p53, or PAb421, which immunoprecipitates both wild-type and mutant p53. Immunoprecipitated proteins were separated by SDS-PAGE, transferred to a membrane, and probed with a sheep anti-p53 poly-

FIG. 5. Identification of the T antigens present in tumors from T1-127-transgenic animals. Proteins (100 μ g) extracted from tumors (#79-75 and #79-78) or from the cell line B6/pPVU0 were immunoprecipitated by the negative control antibody gD or one of the T-antigen-specific antibodies PAb901, PAb416, and PAb419, which recognize epitopes between large T-antigen amino acids 684 and 698, 82 and 127, and 1 and 82, respectively. Immunoprecipitated proteins were subjected to SDS-PAGE, and the proteins were electrotransferred to a PVDF membrane. The blot was probed with PAb419, and immunoreactive proteins were detected by enhanced chemiluminescence. IgG, immunoglobulin G.

DISCUSSION

The results presented here indicate that transgenic mice expressing an N-terminal segment of large T antigen containing amino acids 1 to 127 and expressed under control of the E1 promoter display pancreatic acinar cell dysplasia at birth and microadenomas and acinar cell carcinomas as early as 10 weeks later. By 31 weeks of age, over 90% of the mice in the 79 lineage had developed pancreatic tumors.

The finding that an N-terminal T-antigen segment is sufficient to induce tumors in transgenic animals is consistent with the results of Saenz-Robles et al. (33). These authors showed that a mutant large T antigen consisting of the first 121 amino acids when expressed under control of the LPV promoter was sufficient to induce CPE papillomas. The results reported herein and those involving T1-121 expressed from the LVP promoter differ, however, in two respects. First, the appearance of T1-121-induced CPE tumors was delayed considerably in comparison to CPE tumor development in mice expressing a full-length T antigen (4, 33, 41). Second, inactivation of p53 correlated with progression of the tumors (4, 33, 41). In contrast, the development of pancreatic acinar cell dysplasia and acinar cell carcinomas in mice expressing T1-127 under control of the E1 promoter reported here parallels that observed by others for mice expressing full-length large T antigen (T1-708) and small t antigen under the same promoter (13, 26). Ornitz et al. (26) showed that transgenic mice bearing a transgene that produced both full-length large T and small t antigens under control of the E1 promoter exhibited pancreatic acinar cell hyperplasia at day 17 of fetal development, which after birth progressed to dysplasia and solitary nodule formation between 2 and 10 weeks of age. We showed here that mice expressing an E1-T1-127 transgene had developed acinar cell dysplasia at birth and pancreatic acinar cell carcinomas by 10 weeks. The dysplastic pancreas could be distinguished from normal pancreas by its firmer gross texture and whiter than normal appearance, and histologically by the pleomorphic cells that were arranged in acinar and pseudoacinar formations with enlarged lumen, all very similar to previous descriptions of dysplastic acinar cells (12, 26, 30). In addition to the diffuse pattern of dysplasia seen in newborn animals, we also observed areas of dysplasia in older animals where portions of the dysplastic pancreas had thin bands of fibrous tissue separating groups of acini into vague lobules (Fig. 3A and B). Based on the abnormal development of the acinar cell populations in these animals and the certainty with which they progress to neoplasia, we also consider the tissue to be preneoplastic.

On histological examination, structures which on the basis of

clonal antibody. As controls for antibody specificity, proteins from extracts of the cell line B6/pPVU0 and the cell line B6/C-3 were immunoprecipitated with the same antibodies. As expected (Fig. 6A), p53 was immunoprecipitated from the B6/pPVU0 extract by PAb246 and PAb421 but not by PAb240 or gD, indicating the presence of phenotypically wild-type p53. In contrast, the p53 in the B6/C-3 extract was immunoprecipitated by PAb240 and PAb421 but not by PAb246 or gD, indicating the presence of mutant p53. The p53 in the tumor extracts was immunoprecipitated by PAb246 and PAb421 but not by PAb240. Protein immunoprecipitated by PAb421 from a cell line expressing both wild-type and mutant p53 (lane 1) provided a position marker for p53 in the gel. When twice the amount of tumor extract was used (Fig. 6B) or films were exposed for longer periods (not shown), evidence of mutant p53 still was not found. The band in the lane that received proteins immunoprecipitated from tumor 79-41 extract by gD antibody resulted from spillover from the adjacent lane. No such band appeared when gD was used to immunoprecipitate proteins from the larger amount of tumor extract (Fig. 6B). Identical results were obtained for each of the six additional tumors examined (not shown). These results indicate that the p53 in the tumor cells has the immunological characteristics of wild-type p53.

FIG. 6. Phenotype of p53 in tumors from T1-127-transgenic mice. (A) Proteins (150- μ g aliquots) extracted from B6/pPVU0 or B6/C-3 cells were immunoprecipitated by using an irrelevant antibody (gD) or a monoclonal antibody that recognizes wild-type p53 (PAb246), mutant p53 (PAb240), or both (PAb421). Electrophoresis and immunoblotting were done as described for Fig. 5 except that sheep anti-p53 antibody was used as the probing antibody. (B) Proteins extracted from tumors were immunoprecipitated and analyzed similarly. The first lane contains proteins immunoprecipitated by PAb421 from 100 μ g of protein extracted from a cell line expressing mouse mutant p53.

morphological criteria appear to be apoptotic bodies were observed in areas of acinar cell dysplasia, microadenomas, carcinoma, and metastatic acinar cells within the liver. The relationships among apoptosis, dysplasia, tumor induction, and tumor progression in T-antigen-transgenic mice appear to vary considerably. Increased p53-dependent apoptosis in response to expression of an N-terminal T-antigen fragment (T1-121) versus full-length T antigen has been reported (4, 23, 41) and correlated with abrogation of tumorigenesis (23) or slow tumor growth (4, 33) when T-antigen expression was targeted to lymphocytes or the choroid plexus, respectively. Similarly, expression of full-length T antigen in mammary gland (35) or liver (2) correlated with increased apoptosis, and progression from dysplasia to carcinoma was associated with diminution of the apoptotic response. However, when T antigen was expressed in the prostate, apoptosis levels in prostate carcinomas were not lower than those in low-grade prostatic intraepithelial lesions (36). Detailed comparisons of the apoptotic indexes in dysplastic tissue, in adenocarcinomas, and in metastases from E1-T1-127 mice will be needed in order to determine the impact of apoptosis on the development of acinar cell carcinoma.

Pancreatic tumors from T1-127-transgenic mice were examined for expression of large and small t antigens and for the conformation of p53. In each of these, a mutant large T antigen with the immunological reactivity and size consistent with a protein containing the first 127 amino acids of the protein and small t antigen were observed. The development of tumors did not, therefore, appear to depend on restoration of the large T-antigen reading frame. Thus, the N-terminal T-antigen segment was sufficient for tumors to develop.

The timing of tumor appearance in the E1-T1-127 mice was similar to that reported by others for transgenic mice expressing full-length large T and small t antigens. In individual transgenic lines expressing large T and small t antigens under control of the elastase promoter, the average age to death varied between 12.6 and 28.2 weeks (13, 21, 26). The average age to death of 21.4 weeks for the T1-127 mice is within that range.

It has been reported previously that the genetic background in which a transgene is placed can influence the timing of tumor development (9). The T1-127 mice described here were generated in C57BL/6 $\times$ D2F1 mice. The progeny mice then were crossed with B6 mice. The E1-SV40 T-antigen transgenic lines of mice described previously (26), to which many of the comparisons reported herein were made, were produced in a (C57BL/6 $\times$ SJL) F_1 hybrid mice. Therefore, it remained possible that the timing of tumor formation in the E1-T1-127 mice was delayed relative to tumor formation in (C57BL/6 $\times$ D2) F_1 mice expressing full-length large T and small t antigens. A substantial delay in tumor formation in mice expressing a truncated T antigen relative to full-length T antigen in the same genetic background might suggest the need for additional cellular mutations for tumor induction or progression.

To gain insight into whether the difference in genetic background might play a major role in the relative time of tumor appearance, we generated a transgenic line (E1-T1-708) bearing a full-length large T antigen and small t antigen under transcriptional control of the E1 promoter in the C57BL/6 $\times$ D2F1 genetic background and bred the mice in the same manner as used for the E1-T1-127 mice. The E1-T1-708 mice developed pancreatic acinar cell carcinomas that were indistinguishable histologically (data not shown) from those that developed in the T1-127 and T1-147 transgenic lines. Although studied less extensively than the T1-127 line, over the course of a 31-week observation period, five of seven E1-T1-708 mice developed tumors at times ranging from 12.5 to 30.5 weeks, with a mean time to death among the mice with tumors of 20.8

weeks. As additional mice are examined, the kinetics of tumor development in this genetic background can be defined more precisely. However, based on the data available, it appears that the tumor development in the E1-T1-708 mice is not more rapid than that reported previously for mice expressing full-length large T antigen and small t antigen under control of the elastase promoter or for the E1-T1-127 transgenic line.

All tumors from E1-T1-127-transgenic mice examined, regardless of the tumor size or the age at which the mouse became moribund, contained only p53 with the immunological reactivity expected of wild-type p53. These findings indicate that in acinar cells expressing a T antigen incapable of binding p53, mutational inactivation of the tumor suppressor protein is not needed in order for tumors to develop and progress to life-threatening size. The possibility that the amino acid 1-127 segment of T antigen alters the activity of p53 cannot be ruled out as yet, however.

A notable difference between the T1-127 transgene reported here and the T1-121 transgene reported by Saenz-Robles et al. (33) is that the construct expressing T1-127 expresses small t antigen as well as the N-terminal large T-antigen fragment whereas the T1-121 transgene did not express small t antigen. Small t antigen in cell culture enhances some of the cell culture-transforming properties of large T antigen when the large T antigen is present in limited amounts (3), when cells are quiescent (32, 49), and in specific transformation assays (3, 48). In addition, small t antigen has been reported to enhance tumorigenesis in transgenic animals in slow-growing tissues (8).

The extent to which small t antigen contributes to the generation of tumors in the T1-127-transgenic mice remains to be determined. However, it seems unlikely to play a dominant role. We also generated transgenic mice expressing a T antigen containing amino acids 1 to 147. This T1-147 construct does not produce small t antigen (38, 39). Although fewer of these animals were studied, the results in Table 1 show that these mice also develop acinar cell tumors in a similar time frame as T1-127-transgenic mice do, and the tumors that develop are indistinguishable histologically from those in E1-T1-127 mice. A likely conclusion from these results is that small t antigen is not essential for induction of acinar cell tumors, and its presence does not alter the time frame for tumor development substantially.

Recently an additional T antigen, the 17K T antigen, has been described (51). This protein shares its N-terminal 131 amino acids with large T antigen, followed, by virtue of an RNA splicing event, by four amino acids encoded by an alternate reading frame. The 17K T antigen has the capacity to alter the growth behavior of cells in vitro (51). However, the 17K T antigen is not required for the genesis of the acinar cell carcinomas described herein, as the deletion in the T1-127 T-antigen coding region removes the splice donor for the 17K T-antigen mRNA.

Large T antigen has been examined extensively for its ability to transform cells in culture. Mutational analyses revealed that individual growth behavior changes that collectively constitute the transformed cell phenotype depend on specific T-antigen activities. The T1-127 segment of T antigen contains at least three regions that participate in transformation of cells in culture. Deletion of the N-terminal 127 amino acids of large T antigen abrogates multiple transforming activities. A T antigen missing those amino acids cannot confer on cells the ability to grow to high cell density, to grow rapidly in medium containing low concentrations of serum, or to divide in the absence of anchorage (48). We showed recently (46) that restoration of Rb-binding capacity to an N-terminally truncated T antigen

missing amino acids 1 to 127 restored the capacity to grow to high cell density. However, restoration of rapid growth in low-serum-containing medium and anchorage-independent growth required transforming activities contained within the first 82 amino acids of T antigen (T/t common region). Similarly, mutations within or including the binding site for the Rb family of proteins (Rb/p107/p130), which is encompassed by amino acids 101 to 118, prevent dense focus formation (7, 19, 31) and anchorage-independent growth (7, 48), and removal of the T/t common region prevents T antigen from conferring anchorage-independent growth (25).

Recently, two independent transforming activities within the T/t common region were described (29). One of these is impaired by mutations in the amino acid 17–27 region, and the second is impaired by mutations in the hexapeptide HPD-KGG, which is conserved among papovaviruses (28).

At least two of the T-antigen N-terminal transforming activities affect tumorigenesis in specific cell types in transgenic mice. Mutational inactivation of Rb/p107/p130-binding capacity in the LPV-T1-121 transgene prevents induction of CPE tumors (33). Deletion of amino acids 17 to 27 from T1-708 prevents development of CPE tumors and B-cell lymphomas, whereas the capacity to induce thymic lymphomas is retained (23, 42). How these individual transformation-related activities and the activity that localizes to the hexapeptide region affect tumorigenesis in the pancreas remains to be determined. The use of transgenic mice expressing N-terminal segments of T antigen with defined mutations known to inactivate the individual transforming activities under control of the E1 promoter appears particularly attractive, since tumorigenesis is not compromised by the inability of the T-antigen segment to bind p53.

We showed previously (5) that two segments of T antigen have an analogous transforming activity. The mutant 1-147 and a T antigen containing amino acids 251 to 708 independently provide immortalizing activity that cooperates with an activated *ras* oncogene to produce dense foci on primary rat embryo fibroblast monolayers. T1-147, like T1-127 when expressed from the E1 promoter, induces acinar cell carcinomas. Whether an activity in the T251-708 also is capable of inducing pancreatic neoplasms remains to be determined.

The results presented here and by others indicate that the activities of T antigen needed to initiate tumors depend on the tissue targeted for expression. It will be of considerable interest to determine the spectrum of tumors that can be induced by specific segments of T antigen when fragments of the oncoprotein are expressed from a promoter/enhancer that is capable of expression in a wide variety of cell types. Such documentation would lay a firm foundation for identifying oncoprotein functions that impact on genesis of specific tumor types.

ACKNOWLEDGMENTS

The excellent technical assistance of Holly A. Lacko, Ardell Conn, Melanie Epler, and Rebecca Pound is gratefully acknowledged.

This work was supported in part by Public Health Service grant CA24694 from the National Cancer Institute of the National Institutes of Health.

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