14. R. Korneluk *et al.*, *J. Biol. Chem.* **259**, 13819 (1985).
15. G. Casey, R. Smith, D. McGillivray, G. Peters, C. Dickson, *Mol. Cell. Biol.* **6**, 502 (1986).
16. L. P. Shen and W. J. Rutter, *Science* **224**, 168 (1984).
17. Y. Yuasa, E. P. Reddy, S. J. Rhim, S. R. Tronick, S. A. Aaronson, *Jpn. J. Cancer Res. (GANN)* 77, 639 (1986).
18. R. White *et al.*, *Nature (London)* **318**, 382 (1985).
19. W. K. Cavenee, R. J. Leach, T. Mohandas, P. Pearson, R. L. White, *Am. J. Hum. Genet.* **36**, 10 (1984).
20. M. Jansson, B. Philipson, B. Vennstrom, *EMBO J.* **2**, 561 (1984).
21. K. C. Robbins, S. G. Devare, S. A. Aaronson, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2918 (1981).
22. I. Ali *et al.*, unpublished data.
23. C. Turleau *et al.*, *Hum. Genet.* **67**, 455 (1984); E. E. Michalopoulos *et al.*, *ibid.* **70**, 157 (1985); V. van Heyningen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 8592 (1985); T. Glaser *et al.*, *Nature (London)* **321**, 882 (1986).
24. J. Yokota, Y. Tsunetsugu-Yokota, H. Battifora, C. Le Fevre, M. J. Cline, *Science* **231**, 261 (1986).
25. P. S. Henthorn, D. L. Mager, T. H. J. Huisman, O. Smithies, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5194 (1986).
26. C. Huerre, S. Despoisse, S. Gilgenkrantz, G. M. Lenoir, C. Junien, *Nature (London)* **305**, 638 (1983); B. de Martinville and U. Francke, *ibid.*, p. 641; A. G. Van Kessel, R. Nusse, R. Slater, P. Tetteroo, A. Hagemeijer, *Cancer Genet. Cytogenet.* **15**, 79 (1985); K. Nahara *et al.*, *Hum. Genet.* **66**, 181 (1984).
27. F. Hecht and T. W. Glover, *Cancer Genet. Cytogenet.* **13**, 185 (1984).
28. A. L. Murphree and W. F. Benedict, *Science* **223**, 1028 (1984); W. K. Cavenee *et al.*, *ibid.* **228**, 501 (1985).
29. H. Klinger, *Cytogenet. Cell Genet.* **32**, 68 (1982); E. J. Stanbridge, *BioEssays* **3**, 252 (1985).
30. E. J. Stanbridge *et al.*, *Somatic Cell Genet.* **7**, 699 (1981); W. F. Benedict, B. E. Weissman, C. Mark, E. J. Stanbridge, *Cancer Res.* **44**, 3471 (1984).
31. M. Kaelbling and H. P. Klinger, *Cytogenet. Cell Genet.* **41**, 65 (1986).
32. A. Koufos *et al.*, *Nature (London)* **316**, 330 (1985).
33. C. E. Rogler *et al.*, *Science* **230**, 319 (1985).
34. C. Escot *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4834 (1986).
35. D. J. Slamon *et al.*, *Science* **235**, 177 (1987).
36. I. Ali *et al.*, in preparation.
37. M. Gross-Bellard, P. Oudet, P. Chambon, *Eur. J. Biochem.* **36**, 32 (1973).
38. R. Lidereau *et al.*, *J. Natl. Cancer Inst.* 77, 697 (1986).
39. M. Goldfarb, K. Shimizu, M. Perucho, M. Wigler, *Nature (London)* **296**, 404 (1982).
40. A. Grzeschik and H. H. Kazazian, *Cytogenet. Cell Genet.* **40**, 180 (1985).
41. We thank G. Bell, W. Cavenee, E. Chang, C. Dickson, T. Holm, H. Kazazian, T. Korneluk, B. Nelkin, S. Tronick, G. Vande Woude, and B. Vennstrom for various probes used in this study. We are grateful to G. Smith and J. Kantor for helpful comments on the manuscript and to M.-H. Champeme for excellent technical help.

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Pancreatic Neoplasia Induced by SV40 T-Antigen Expression in Acinar Cells of Transgenic Mice

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Three lines of transgenic mice were produced that develop pancreatic neoplasms as a consequence of expression of an elastase I–SV40 T-antigen fusion gene in the acinar cells. A developmental analysis suggests at least a two-stage process in the ontogeny of this disease. The first stage is a T antigen–induced, preneoplastic state characterized by a progression from hyperplasia to dysplasia of the exocrine pancreas, by an increased percentage of tetraploid cells, and by an arrest in acinar cell differentiation. The second stage is characterized by the formation of tumor nodules that appear to be monoclonal, because they have discrete aneuploid DNA contents. The cells within the nodules as compared to normal pancreatic tissue have less total RNA by a factor of 5, less pancreas-specific messenger RNA by a factor of about 50, and increased levels of T-antigen messenger RNA. A tumor cell line has been derived that retains both pancreatic and neoplastic properties.

CARCINOMA OF THE PANCREAS IS the fourth leading cause of death due to cancer in the U.S. population, and the prognosis, once detected, is particularly grim (*1*). Currently there is no adequate animal model for this condition. Previous studies have shown that it is possible to introduce foreign DNA into the germline of mammals by injecting DNA directly into the pronucleus of fertilized eggs and then transferring the eggs to foster mothers so that development can continue (*2*). The resulting transgenic animals generally carry one or more copies of the foreign DNA integrated into one of their chromosomes. An application of this technique has been to produce transgenic animals carrying foreign genes that predispose them to cancer (*2, 3*). Because the foreign genes are transmitted in a normal Mendelian manner, it is possible to mimic dominant hereditary cancers by producing lines of mice in which all of the offspring that inherit the transgenes succumb to cancer. Moreover, by fusing the regulatory regions (enhancers) from genes that are known to be expressed in a tissue-specific manner to transforming genes, it is possible to direct expression of the transforming genes to specific cell types and thereby produce lines of mice that develop tumors only in specific organs (*2–4*).

To develop an animal model for pancreatic cancer, we have made transgenic mice in which the transforming gene from the simian virus 40 (SV40) genome is placed under the control of the regulatory elements from the rat elastase I gene. Elastase is one of several pancreatic serine proteases that is synthesized in the exocrine cells and secreted into the gut. Expression of elastase normally commences at about day 14 of development into the mouse, when the acinar cells begin to differentiate. The levels of elastase I messenger RNA (mRNA) and protein increase dramatically during the next few days, and they plateau a few weeks after birth (*5*). The promoter and enhancer of the rat elastase I gene lie within the 205 bp 5′ of the transcription start site (*6, 7*). When they are fused to the human growth hormone (hGH) structural gene, transgenic mice bearing this construct synthesize hGH exclusively in pancreatic acinar cells. Furthermore, the rate of transcription of the transgene is comparable to that of the endogenous elastase gene, and it is activated appropriately at day 14 of fetal development (*7, 8*). On the basis of these results, we have used the elastase promoter and enhancer to direct the expression of several potential transforming genes to pancreatic acinar cells. Unlike the fetal tumors that develop when an activated *ras* gene is expressed in pancreatic acinar cells (*9*), when the SV40 early region [which codes for small and large T antigens (*10*)] is placed under the control of the elastase regulatory elements, tumors develop in the adult. This is presumably a consequence of secondary genetic or epigenetic events.

Four transgenic mice bearing both elastase-SV40 early region (ELSV) and elastase-neomycin (ELNEO) fusion genes were produced by microinjection of linear DNAs containing these genes into pronuclei of fertilized eggs (Fig. 1). All of the founder mice died of pancreatic cancer by 6.5 months of age; however, three transgenic lines of mice were established by breeding the founders prior to overt tumor formation (Table 1). We examined 127 transgenic mice from these lines that were more than 3 months of age, and all of them either died with pancreatic tumors or contained obvious tumor nodules when killed. Tumor de-

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velopment in these lines is faster than that described previously for the 177-5 line (Table 1). In these three lines, the pancreas of newborn mice is obviously hyperplastic at 2 weeks of age and by 10 weeks many nodules appear, which continue to expand into a tumor mass (Fig. 2).

To ascertain whether the ELSV transgene was expressed during the fetal and neonatal periods, histological sections of pancreas were immunologically stained for T antigen. Most transgenic mice expressed detectable levels of T antigen by day 17 of gestation, and all of them expressed T antigen by 5 days after birth. In mice that express T antigen at day 17, T antigen was located in the nucleus of all acinar cells but not in other cells (Fig. 3, A and B). Histological sections of pancreas at this stage revealed a relatively normal tissue architecture, with the exocrine cells growing in acinar arrangements surrounding scattered clusters of normal islet cells. However, in transgenic mice the acini had a much higher cell density and smaller acinar cell size than those of normal littermates (Fig. 3, C and D). In addition, the mitotic activity was increased from about 0.5 mitosis per high power field in control to about 10 in transgenic pancreas. Throughout the perinatal period the pancreas acquired a dysplastic phenotype, characterized by increasingly disorganized acini and the presence of cells with enlarged, irregular nuclei, containing multiple nucleoli. On a macroscopic level the pancreas was markedly enlarged, the texture was firm, and the color was whiter than normal (Fig. 2A). This phenotype was apparent as early as day 17 of gestation.

Transgenic mice between 2 and 10 weeks of age developed many solitary nodules embedded in a dysplastic pancreas (Fig. 2, A and B). On histological examination, the nodules contained pleomorphic cells growing in pseudoacinar structures with enlargement of the lumina and the formation of cystic and dilated vascular spaces. No islet tissue was present within these solitary nodules (Fig. 3, E and F). Some cells had giant, irregularly shaped, hyperchromatic nuclei that contained multiple nucleoli (Fig. 4, B to D). Immunocytochemical detection of large T antigen in these early nodules revealed an increased staining intensity compared to surrounding tissue (Fig. 3, F and G). From analyzing many sections of transgenic pancreatic tissue, we estimate that hundreds of tumor nodules form during the progression of this disease.

Further progression of pancreatic tumors was characterized by a large increase in the size of the tumor nodules. The nodules showed increasing pleomorphism of the cells, which grew in sheets as well as in occasional pseudoacinar structures. The nodules contained large cystic and vascular spaces and areas of central necrosis (Figs. 3H and 4, C and D). Many of the nodules also formed a fibrous capsule, probably derived from compressed pancreatic stromal cells. However, even though the nodules grew together to form a massive tumor (up to 30% of the weight of the mouse), relatively normal appearing regions of exocrine pancreas could be found at the edge of large nodules (Fig. 3H) that appeared histologically similar to the hyperplastic or dysplastic cells that were observed at earlier stages of development. Staining these sections for T antigen revealed more T antigen in the nodule cells than in the preneoplastic cells (Fig. 3I), although some dysplastic cells had levels of T antigen comparable to that seen in nodule cells. In most animals that have been autopsied, the tumor was contained within defined boundaries. We have rarely (2 of 127 mice) observed metastasis into

Table 1. Elastase-SV40 T-antigen transgenic lines of mice. Transgenic mice were produced by microinjecting linearized DNA molecules into the pronucleus of fertilized C57/SJL F2 hybrid eggs (*18*). In the first experiment, seven transgenic mice were produced and they all died of pancreatic tumors by 4 months of age; one line (177-5) was established (*8*). In the second experiment, four transgenic mice were produced and they all died with pancreatic tumors by 6.5 months; three lines were established. Offspring from these lines were used to establish the average age at death, neonatal expression of T antigen (determined by immunocytochemistry), and transgene copy number (*8*).

Transgenic lines	No. of transgenic offspring	Transgene copy no.	Age at death (weeks)	Neonatal expression of T antigen
177-5	31	14	28.2 ± 9.4*	No
264-4	12	8	18.5 ± 3.6	Yes
266-5	15	17	14.6 ± 5.2	Yes
266-6	15	3	12.6 ± 2.6	Yes

*SD.

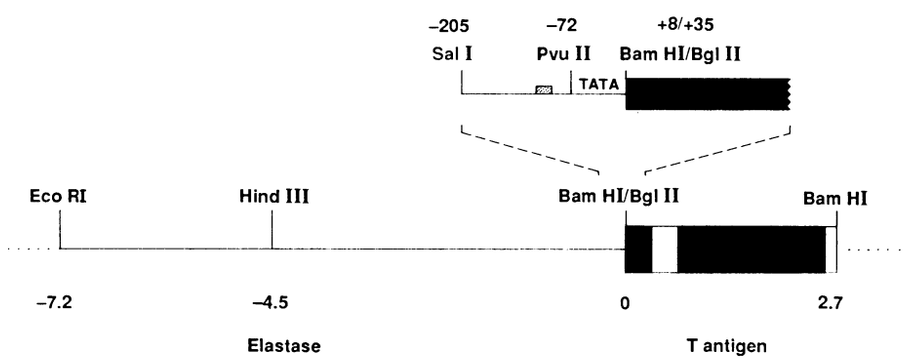

Fig. 1. The elastase-SV40 T-antigen fusion gene introduced into mice. The rat elastase I–SV40 T-antigen fusion gene contains 7.2 kb of rat elastase I 5′ flanking DNA fused to the SV40 T-antigen gene at position +35. The Stu I site at position 5190 on the standard SV40 map was converted to a Bgl II site with a synthetic linker. The 5′elastase I regulatory region with a synthetic Bam HI linker at position +8 was constructed as previously described (*6*) and fused to the Bgl II linker in the SV40 T-antigen gene. The 3′ end of the fusion gene extends to the Bam HI site at position 2533 in SV40. The tissue-specific elastase enhancer is contained between the Sal I site at position −205 and the Pvu II site at position −72. The stippled box represents the location of a sequence that was conserved in the promoter region of elastase and other serine protease genes. The elastase-NEO fusion gene contains 4.5 kb of elastase 5′ sequence fused to the bacterial Tn5 neomycin phosphoribosyltransferase gene. The elastase 5′ sequence replaces the SV40 promoter in pSV2NEO (*19*). Linear DNA restriction fragments containing the fusion genes were purified by agarose gel electrophoresis. Several hundred molecules of each gene were injected into fertilized mouse eggs (*18*).

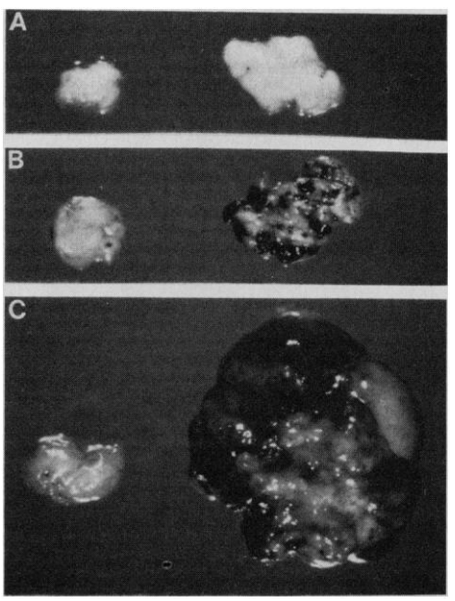

Fig. 2. Gross appearance of pancreas from transgenic and normal littermates at (**A**) 2, (**B**) 10, and (**C**) 15 weeks. Left column, the pancreas from age-matched littermates; right column, transgenic pancreas from the 266-6 line.

kidney and lung, although seeding into the peritoneal cavity was relatively common. Because a large number of nodules develop rapidly and simultaneously, there may be insufficient time for metastasis to occur before the mice die.

In normal pancreas from 2-week-old mice, about 90% of the cells had a diploid DNA content as measured by flow cytometry, which suggests that they were in either G_1 or G_0 phase of the cell cycle. About 3% of the cells had a G_2/tetraploid DNA content, and 7% of the cells were in S phase. Transgenic littermates at 2 weeks of age showed a fourfold increase in the proportion of cells with a G_2/tetraploid DNA content and a twofold increase in S-phase cells compared to age-matched controls. The number of pancreatic cells in control mice increased from about 3×10^6 at birth to about 9×10^7 at 4 weeks of age (assuming that all cells are diploid and contain 6.4 pg of DNA) The total DNA content of the transgenic pancreas was three- to sevenfold greater than that of age-matched controls during this period.

In normal adult pancreatic tissue, about 93% of the cells had 2N (diploid) DNA content, about 6% of the cells had a G_2/tetraploid DNA content, and about 1% of the cells appeared to be in S phase (cells between the 2N and 4N peaks, Fig. 4A). Comparing these cell-cycle parameters with those of dysplastic pancreatic tissue from a 13-week-old mouse (Fig. 4B) shows a striking (up to 18-fold) increase in the number of cells with a G_2/tetraploid DNA content. Both the diploid and tetraploid cells appeared to be participating in the cell cycle as indicated by a 25-fold increase in the number of nuclei with DNA content between the 2N and 4N peaks and the 4N and 8N peaks (Fig. 4B).

Seven nodules from three mice representing two transgenic lines were also analyzed by flow cytometry. Most of the nuclei were aneuploid in these tumor nodules (Fig. 4, C and D). Four individual tumor nodules from the same mouse contained discrete aneuploid peaks with DNA contents that varied from 2.3N to 2.8N, which suggests

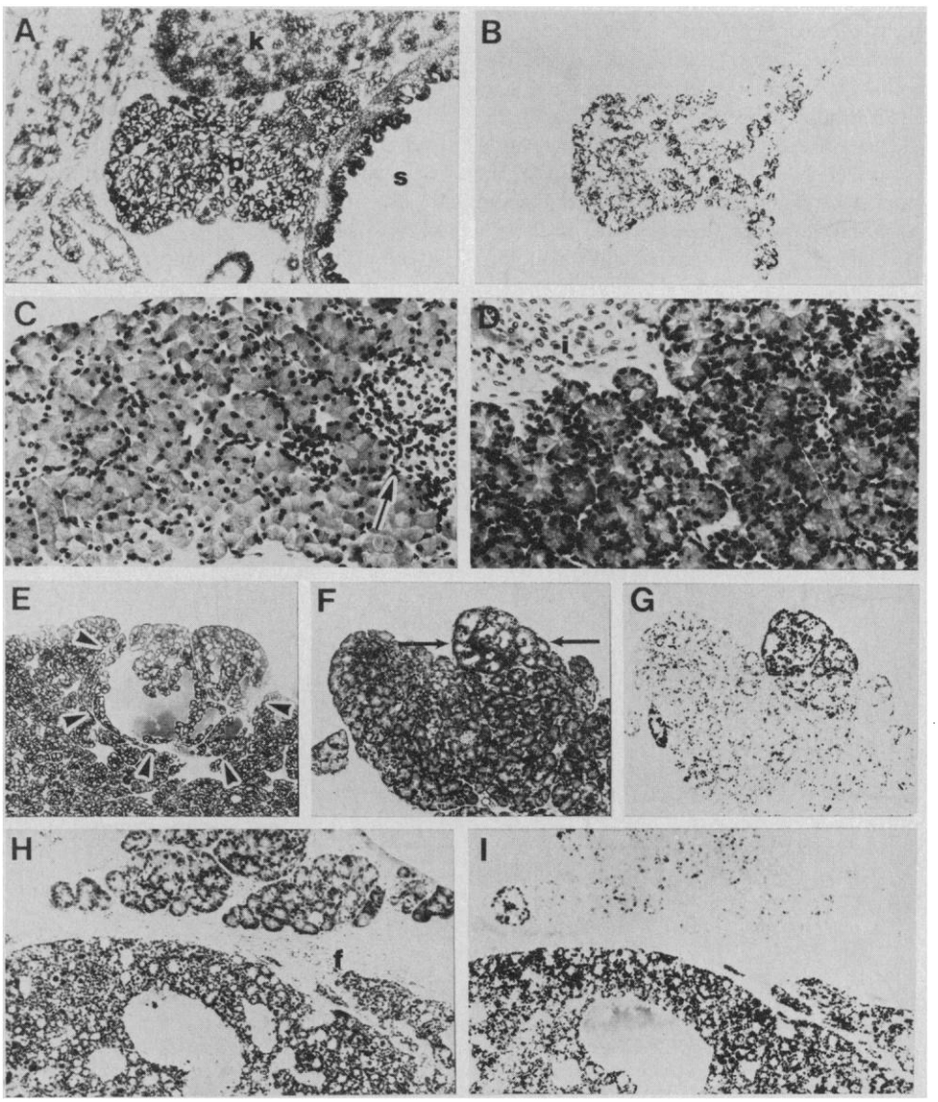

Fig. 3. Developmental histopathology and immunohistochemical detection of large T antigen in T antigen–induced pancreatic tumors. All photographs were of tissues from mice in the 264-4 line. Tissue samples were fixed in Bouin's fixative, washed in 70% ethanol, and embedded in paraffin. Some fetal stages were examined by snap-freezing of the whole body in liquid nitrogen followed by cryosectioning at 10 mm. Staining and immunocytochemistry were performed essentially as described (*8*), with the avidin-biotin-peroxidase procedure (*20*) and monoclonal antibody PAb 101, previously named 412 (*21*). (**A**) View of the pancreas in a whole-body frozen section (10 mm) of a 17-day fetus. Hematoxylin and eosin (H&E)–stained, magnification ×38. Abbreviations: k, kidney; s, stomach; and p, pancreas. (**B**) Section adjacent to (A), immunoperoxidase-stained with monoclonal antibody PAb 101 against large T antigen. All of the exocrine cells show positive nuclear staining. The clear areas within the pancreas represent blood vessels, interlobular stroma, and islets. No staining was seen in the surrounding tissues. Magnification ×38. (**C**) H&E–stained paraffin section of the pancreas of a normal 18-day fetus. A large islet (arrow) is present at the right side of the field. Magnification ×192. (**D**) H&E–stained paraffin section of the pancreas of a transgenic 18-day fetus, shown at the same magnification as its normal littermate in (C). There is an increased cell density throughout all of the exocrine pancreas, with the cells still growing in acinar arrangements. A normal islet (i) is present at the upper left corner of the field. (**E**) Low-power view of an H&E–stained paraffin section of the pancreas from a 59-day-old transgenic mouse. The section contains a solitary nodule shown in the center of the field, which compresses the surrounding pancreas. The margins of the nodule are indicated by arrowheads. Note the large cystic and dilated vascular spaces within the nodule. Magnification ×29. (**F**) H&E–stained paraffin section of an early nodule (indicated by arrows) forming at the edge of the pancreas of a 19-day-old transgenic mouse. Magnification ×70. (**G**) Section adjacent to (F), immunoperoxidase-stained with monoclonal PAb 101 against large T antigen. The nodule appears to stain more intensely than the majority of cells in the surrounding pancreas. A smaller intensely staining nodule is seen in the lower left that is not apparent in the H&E section. Magnification ×70. (**H**) H&E–stained paraffin section of pancreas from a 152-day-old transgenic mouse. The edge of an advanced nodule is shown at the bottom of the field, separated by a fibrous capsule (f) from residual compressed dysplastic pancreas at the top. The advanced nodules are composed of cells growing in sheets with little acinar organization and with several cystic or dilated vascular spaces. Magnification ×96. (**I**) Section adjacent to (H), immunoperoxidase-stained with monoclonal PAb 101 against large T antigen. The cells in the advanced nodule continue to stain more intensely than the majority of the cells in the compressed dysplastic pancreas at the top of the field. Magnification ×96.

that the cells within an individual nodule may be karyotypically similar and that the nodules are monoclonal in origin. The aneuploid cells also appeared to be dividing at a high rate as evidenced by the large number of nuclei with a DNA content between the aneuploid G_1 and G_2 peaks (Fig. 4, C and D). The diploid cells in these samples probably represent compressed pancreatic stromal cells that form a fibrotic capsule as well as vascular endothelial cells and lymphocytes.

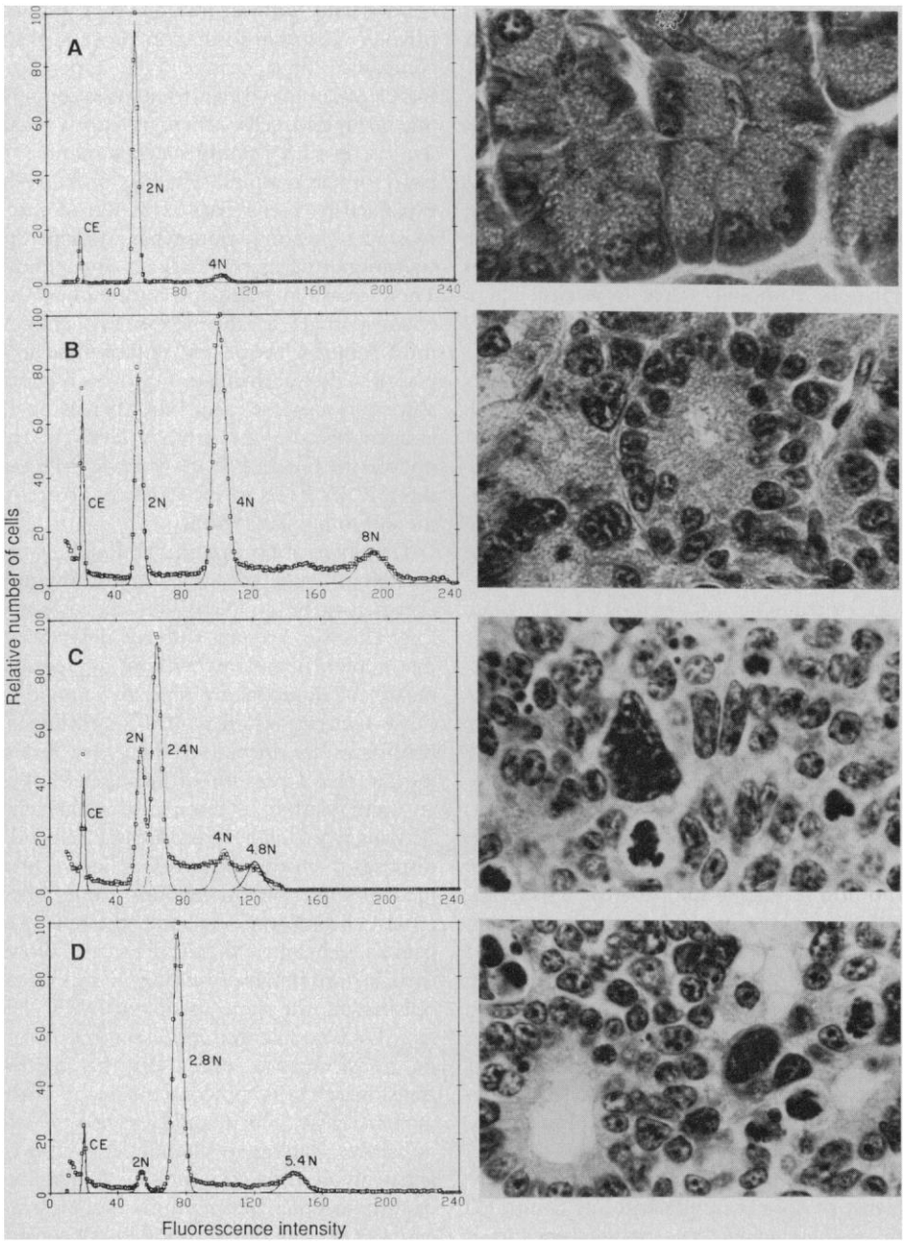

Fig. 4. Flow cytometry and histology of pancreas at various stages of neoplastic transformation. Left panels show flow cytometry analysis of pancreas. Tissue samples were minced in a saline buffer containing 0.1*M* tris (*p*H 7.4), 0.1% NP-40 (Sigma), 2 m*M* $CaCl_2$, 21 m*M* $MgCl_2$, and 10 mg 4.6-diamidino-2-phenylindole (DAPI) per milliter of buffer. Nuclear clumping was reduced by several passages through a 25-gauge needle. Flow cytometry was performed on a Becton Dickinson FACS analyzer. Emission was detected above 400 nm. Data were collected and analyzed on a DEC LSI 11/23 computer (Digital Equipment Corporation) as described (*17*). DNA content was measured relative to chicken erythrocytes (CE); 2N, 4N, and 8N peaks represent normal diploid cells and cells with tetraploid or octaploid DNA content. Other peaks represent cells with aneuploid DNA content. Right panels show corresponding H&E–stained sections of pancreatic tissue used for flow cytometry. All histology was at the same magnification, about ×620. (**A**) Normal adult pancreas with a predominantly diploid population of cells. (**B**) Dysplastic ELSV transgenic pancreas from the 264-4 line, showing a major tetraploid population of cells and a large number of S-phase cells (cells between the major peaks). Histological example of dysplastic pancreas, showing densely packed nuclei and some abnormal nuclei. (**C**) and (**D**) Neoplastic ELSV nodules (264-4 line) containing major unique aneuploid populations of cells. Histological example of aneuploid cells showing abnormal cells, nuclei, and decreased cytoplasmic staining.

Quantitative DNA dot hybridization analysis of dissected tumor nodules and preneoplastic pancreatic tissue showed that the ELSV transgene copy number increased by about 1.6-fold relative to the endogenous metallothionein gene in nodules of most mice. This slight increase may be a consequence of the aneuploidy of the nodules.

During postnatal development of the pancreas, the levels of mRNA coding for digestive enzymes increase dramatically (*5*). As a consequence of this massive commitment to secretory protein synthesis, the RNA-to-DNA ratio of the normal adult pancreatic tissue is higher than that of most other tissues. To follow the effect of T-antigen expression on acinar cell differentiation, we measured the RNA-to-DNA ratio, the level of the pancreas-specific elastase I mRNA, as well as the level of T-antigen mRNA, throughout development. In normal pancreas, an adult RNA-to-DNA ratio of 8 was reached by 30 days (Fig. 5A). The RNA-to-DNA ratio in the pancreas of two ELSV transgenic lines rose more slowly, peaked at a ratio of 2 by 20 days of age, and then fell to a ratio of about 1 in the mature tumor. Elastase mRNA levels increase approximately 100-fold after birth and reach about 10,000 molecules per cell in the normal adult (*5*). In ELSV transgenic mice, the level of elastase mRNA peaked by 30 days of age and then decreased as the tumors developed, which suggested that the transformed phenotype was incompatible with normal pancreatic maturation (Fig. 5B).

In line 264-4, there were only 5 to 10 T-antigen mRNA molecules per cell in the dysplastic stage; this number increased about fivefold in the mature tumor (Fig. 5C). In the 266-6 line, T-antigen mRNA levels were initially about 50 molecules per cell and also increased approximately fivefold in the mature tumor. Quantitation of T-antigen mRNA levels in tumor nodules versus surrounding pancreatic tissue revealed about a threefold increase in mRNA levels in the nodule tissue. This was consistent with the increased immunocytochemical staining observed in nodule tissue (Fig. 3, G and I).

The ability to direct tumorigenesis to specific cell types suggested the possibility of generating transformed cell lines that maintain a partially differentiated phenotype. Cells from an ELSV pancreatic tumor (266-6 line) were placed in culture and maintained for more than a year. These cells expressed high levels of T-antigen mRNA (~700 molecules per cell) and protein. They were also resistant to the drug G418, indicating expression of the ELNEO transgene. This gene may allow selection of cells ex-

pressing a pancreatic phenotype in future experiments. The level of elastase mRNA was about 2% of that of adult pancreas.

Four F_1 C57 × SJL syngeneic mice were injected intraperitoneally with the cultured cells to assess their tumorigenicity. In the first experiment, two animals were injected with 5×10^6 cells. One mouse developed a subcutaneous tumor near the site of injection and the other mouse developed a large peritoneal tumor mass. Histological examination of these tumors revealed a solid disorganized mass of cells. The intraperitoneal tumor contained occasional acini located within the tumor mass as well as clusters of peripherally located cells with some acinar structure. This phenomenon was not observed in the subcutaneous tumor. In the second experiment, two mice were injected intraperitoneally with 10^5 cells. One of the mice developed a subcutaneous tumor after 6 weeks while the second animal had no apparent pathology after 6 weeks. These tumors all contained SV40 DNA and expressed T-antigen mRNA.

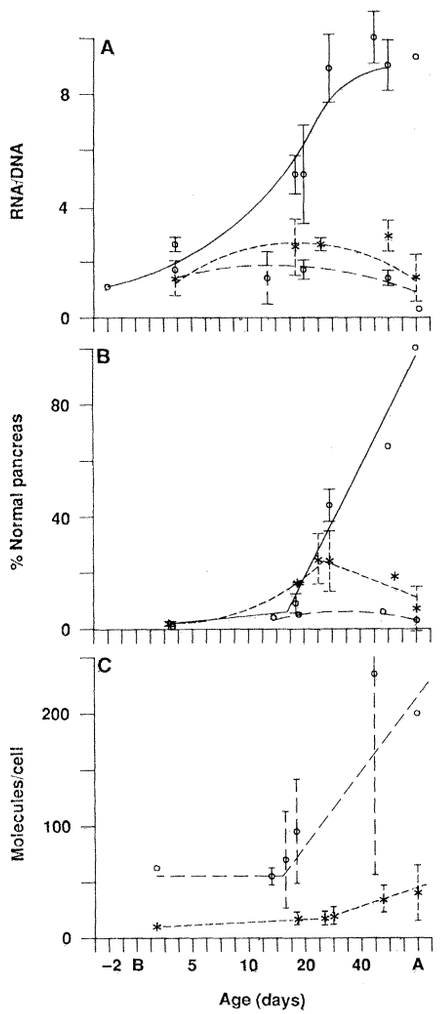

Fig. 5. RNA levels in developing mouse pancreas. About 50 mg of tissue was homogenized in 4 ml of SET [1% SDS, 5 m*M* EDTA, and 10 m*M* tris-Cl (*p*H 7.5)] with 100 μg of proteinase K per milliliter of solution. Total nucleic acids (TNA) were purified as previously described (*22*) and stored frozen in 0.2× SET. DNA concentrations were measured with a fluorescent dye–binding assay (*23*). TNA concentrations were determined by measuring absorbance at 260 nm on a Beckman spectrophotometer. T-antigen mRNA was quantitated with an RNA probe synthesized in vitro with [^{32}P]uridine 5′-triphosphate (*24*). The complementary RNA probes were hybridized with TNA and quantitated as described (*25*). Elastase mRNA was quantitated by a solution hybridization procedure with an end-labeled, 25-base oligonucleotide probe (*3*). (**A**) Ratio of RNA to DNA. (**B**) Mouse elastase I mRNA. (**C**) T-antigen mRNA. Control pancreas (solid line and open circles) and transgenic pancreas (short dashed line and asterisks, 264-4; long dashed line and open circles, 266-6). Curves have been added to help the reader visualize the phenomena. They are not intended to represent statistically fitted curves. Error bars are SD; n = 1 to 4.

In summary, we have described three lines of transgenic mice that contain elastase-SV40 T-antigen fusion genes and develop characteristic tumors of the exocrine pancreas. This phenotype is inherited in an autosomal dominant fashion, and all transgenic offspring die of pancreatic adenocarcinoma at a predictable time (Table 1). The advantage of studying oncogenesis in transgenic mice is that the primary genetic lesion is known. Although the molecular mechanisms involved in SV40 T antigen–induced transformation remain to be elucidated, the pathological changes occur in a predictable fashion.

As early as day 17 of fetal development, transgenic fetuses can be distinguished from their normal littermates by the increased size and firm texture of the pancreas. Histologically, the tissue appears hyperplastic, with increased mitotic activity and an increased cell density of the acini. Flow cytometry showed that more cells were in S phase, and tetraploid cells were much more abundant. During postnatal development the acinar structure deteriorates, and increasing numbers of morphologically abnormal cells accumulate. Although all the acinar cells expressed T antigen, they maintained a semblance of normal architecture and continued to produce acinar cell–specific gene products. Because neoplasms predictably develop in this tissue, we regard it as preneoplastic. In contrast to these lines, T antigen is undetectable in the 177-5 line during the neonatal period, and the cells look completely normal (*8*). Subsequently, a small number of acinar cells begin to express T antigen; they proliferate and ultimately form tumors similar to those described here. The delayed activation of T-antigen expression in only a fraction of the acinar cells accounts for the slower onset of tumor formation in the 177-5 line.

The next stage, in the lines described here, is characterized by a neoplastic transformation of the pancreas. Commencing at about 1 month, multiple nodules develop that display disorganized, rapid cell growth without normal acinar architecture. There is a decrease in differentiated gene products concomitant with an increase in T-antigen mRNA. The transformation process is accompanied by a change from a predominance of tetraploid cells to nodules containing aneuploid cells. There is also a small increase in ELSV transgene copy number in many of the nodules, which is most easily explained by preferential retention of chromosomes bearing transgenes during the transition from tetraploidy to aneuploidy. The increase in transgene copy number accounts partially for the increase in T-antigen mRNA during the progression of these neoplasms. The transformation process is probably not a consequence of increasing T antigen because the mRNA levels in the nodules of the 264-4 line were lower than the mRNA levels in the dysplastic stage of the 266-6 line (Fig. 5C).

The observation that all the nodules were aneuploid suggests that transformation events may be coupled with chromosome loss. This is in keeping with the observation that benign neoplasms without appreciable malignant potential are typically euploid in DNA content, whereas 90% of malignant neoplasms are aneuploid (*11*). Our results indicate that expression of T antigen leads to an accumulation of tetraploid acinar cells. Perhaps rapid replication of these cells predisposes them to chromosomal loss. Chromosomes may be lost randomly, but, when critical chromosomes are lost, the cells may gain an added growth advantage that allows them to proliferate, resulting in a clone of cells having the same aneuploid DNA content. Each nodule thus represents a neoplastic transformation event. In this model, transformation is the consequence of chromosomal loss, which could create a genetic imbalance leading to altered regulation of genes involved in growth control. The loss of chromosomes bearing tumor-suppressing genes (*12*) is an obvious possibility considering the recent evidence regarding the etiology of retinoblastomas (*13*) and Wilms' tumors (*14*). Because hundreds of nodules develop in the hyperplastic pancreas of 1-month-old ELSV mice that contain ~ 7×10^8 cells, it seems unlikely that mutational activation of dominant oncogenes is the critical event, unless the mutation frequency is very high or there is a large number of proto-oncogenes that can be activated and that cooperate with T antigen in neoplastic transformation.

We have established a genetic model for exocrine pancreatic tumorigenesis. Because

acinar and ductal cells may derive from a common precursor, these transgenic mice should serve as a useful model even though the majority of human pancreatic tumors are diagnosed to be of ductal origin (*1*). In addition, the progression of these murine pancreatic tumors resembles several human hereditary malignancies, for example, familial adenomatous polyposis coli (*15*), and the multiple endocrine neoplasia syndromes (*16*). In each of these genetic diseases as well as other malignancies, such as Barrett's syndrome in the esophagus (*17*), adenomas characterized by hyperplasia and normal cellular DNA content become more dysplastic and eventually develop into carcinomas; malignant transformation is associated with the appearance of tetraploid and aneuploid cells. Thus, these ELSV transgenic lines may also provide a useful model for studying progression of other human neoplasms with similar properties.

REFERENCES AND NOTES

1. P. H. Patel and E. Thomas, *Hosp. Pract.* **22**, 131 (1987).
2. R. D. Palmiter and R. L. Brinster, *Annu. Rev. Genet.* **20**, 465 (1986); R. L. Brinster and R. D. Palmiter, *Harvey Lect.* **80**, 1 (1986).
3. D. Hanahan, in *Oncogenes and Growth Control*, T. Graf and P. Kahn, Eds. (Springer-Verlag, Heidelberg, 1986), pp. 349–363.
4. ———, *Nature (London)* **315**, 115 (1985); J. M. Adams *et al.*, *ibid.* **318**, 533 (1985); K. A. Mahon *et al.*, *Science* **235**, 1622 (1987).
5. W. J. Rutter *et al.*, *J. Cell. Physiol.* **72** (suppl. 1), 1 (1968); J. H. Han, L. Rall, W. J. Rutter, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 110 (1986); R. J. MacDonald *et al.*, *Ann. N.Y. Acad. Sci.* **478**, 131 (1986).
6. D. M. Ornitz *et al.*, *Nature (London)* **313**, 600 (1985).
7. R. E. Hammer *et al.*, *Mol. Cell. Biol.* **7**, 2956 (1987).
8. D. M. Ornitz *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **50**, 399 (1985).
9. C. J. Quaife *et al.*, *Cell* **48**, 1023 (1987).
10. J. Tooze, Ed., *The Molecular Biology of Tumor Viruses*, vol. 3 of *Cold Spring Harbor Monograph Series* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ed. 2, 1980).
11. M. F. O'Hara, W. M. Carlos, C. W. M. Bedrossian, T. S. Johnson, B. Barlogie, *Prog. Clin. Pathol.* **9**, 135 (1984); B. Barlogie *et al.*, *Am. J. Med.* **69**, 195 (1980); M. L. Friedlander, D. W. Hedley, I. W. Taylor, *J. Clin. Pathol.* **37**, 961 (1984).
12. E. J. Stanbridge *et al.*, *Science* **215**, 252 (1982); A. G. Geiser, C. J. Der, C. J. Marshall, E. J. Stanbridge, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5209 (1986); P. J. Saxon, E. S. Srivatsan, E. J. Stanbridge, *EMBO J.* **5**, 3461 (1986).
13. W. K. Cavenee *et al.*, *Nature (London)* **305**, 779 (1983); W. K. Cavenee *et al.*, *Science* **228**, 501 (1985); S. H. Friend *et al.*, *Nature (London)* **323**, 643 (1986); W.-H. Lee *et al.*, *Science* **235**, 1394 (1987).
14. B. E. Weissman *et al.*, *Science* **236**, 175 (1987).
15. R. C. Haggitt and B. J. Reid, *Am. J. Surg. Pathol.* **10**, 871 (1986).
16. L. M. Brunt and S. A. Wells, Jr., *Invest. Radiol.* **20**, 916 (1985); T. C. Hsu, S. Pathak, N. Samann, R. C. Hickey, *J. Am. Med. Assoc.* **246**, 2046 (1981).
17. B. J. Reid, R. C. Haggit, C. E. Rubin, P. S. Rabinovitch, *Gastroenterology* **93**, 1 (1987).
18. R. L. Brinster, H. Y. Chen, M. E. Trumbauer, M. K. Yagle, R. D. Palmiter, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4438 (1985).
19. P. J. Southern and P. Berg, *J. Mol. Appl. Genet.* **1**, 327 (1982).
20. S. M. Hsu, L. Raine, H. Fanger, *J. Histochem. Cytochem.* **29**, 577 (1981).
21. E. G. Gurney *et al.*, *J. Virol.* **57**, 1168 (1986).
22. D. M. Durnam and R. D. Palmiter, *Anal. Biochem.* **131**, 385 (1983).
23. C. Labarca and K. Paigen, *ibid.* **102**, 344 (1980).
24. D. A. Melton *et al.*, *Nucl. Acids Res.* **12**, 7035 (1984).
25. L. S. Mathews, G. Norstedt, R. D. Palmiter, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9343 (1986).

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Clonal Analysis of Human Colorectal Tumors

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The clonal composition of human colorectal tumors was studied by means of restriction fragment length polymorphisms (RFLPs). First, X-linked RFLPs were used to examine the pattern of X chromosome inactivation in colorectal tumors of females. All 50 tumors examined showed monoclonal patterns of X chromosome inactivation; these tumors included 20 carcinomas as well as 30 adenomas of either familial or spontaneous type. Second, RFLPs of autosomes were used as clonal markers to detect the somatic loss or gain of specific chromosomal sequences in colorectal tumors. Among other changes, it was found that somatic loss of chromosome 17p sequences occurred in over 75 percent of the carcinomas examined, but such loss was rare in adenomas. These data support a monoclonal origin for colorectal neoplasms, and suggest that a gene on the short arm of chromosome 17 may be associated with progression from the benign to the malignant state.

ALTHOUGH THE ETIOLOGY AND mechanisms of development of human cancer are largely unknown, all hypotheses must incorporate observations concerning the clonality of neoplastic cell populations. Mutational theories of carcinogenesis predict that neoplasms will have a monoclonal composition (*1*). Alternative mechanisms for tumor formation, such as those involving aberrant differentiation processes or field effects, could lead to neoplasms with a polyclonal composition (*2*). At present, knowledge of the clonality of human tumors is incomplete. Many tumors, particularly those of hemopoietic or lymphopoietic origin, have been shown to be monoclonal (*3*), while other tumors appear to be polyclonal (*4–6*).

Colonic neoplasms provide a nearly ideal system to investigate the clonal composition of human cancer. Neoplasms of all stages can be readily identified histologically, from very small benign adenomas to large invasive carcinomas. Furthermore, colonic tumors occur in both heritable and spontaneous forms. Previous studies with isoenzymes of glucose-6-phosphate dehydrogenase (G6PD) have shown that one colorectal carcinoma (*5*) and seven colonic adenomas (*6*) were polyclonal. However, the possible presence of nonneoplastic stromal and epithelial elements within tumors complicates the interpretation of these studies.

We have examined the clonal composition of human colorectal tumors with the aid of two technological advances. First, we used DNA polymorphisms rather than protein polymorphisms. Our rationale was that each normal and neoplastic cell contributes approximately the same amount of DNA; this situation is unlike that potentially obtained by isozyme analysis (for example, G6PD) where the contribution from neoplastic and nonneoplastic cells may be disproportionate. Second, DNA was prepared from cryostat sections of tumors (*7, 8*). Histopathological analysis of alternate cryostat sections provided a reliable estimate of the relative contributions of neoplastic and nonneoplastic cells to the tissue component (that is, DNA) analyzed.

Restriction fragment length polymorphisms (RFLPs) were used to study X chromosome inactivation in colorectal carcinomas from 20 females; representative results are shown in Fig. 1. Analysis of DNA from normal colonic mucosa of a female heterozygous for an RFLP within the phosphoglycerate kinase (PGK) gene (*9, 10*) demonstrated two polymorphic alleles, designated 1 and 2, after Bst XI and Pst I digestion

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