Reduction to Homozygosity of Genes on Chromosome 11 in Human Breast Neoplasia

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The somatic loss of heterozygosity for normal alleles occurring in human tumors has suggested the presence of recessive oncogenes. The results presented here demonstrate a loss of heterozygosity of several genes on chromosome 11 in primary breast tumors. Restriction fragment length polymorphism analysis of these DNAs further suggests that the most frequent loss of sequences in breast tumors occurs between the β-globin and parathyroid hormone loci on the short arm of chromosome 11. The loss of heterozygosity for chromosome 11 loci has a significant association with tumors that lack estrogen and progesterone receptors, grade III tumors, and distal metastasis.

CHROMOSOMAL ABERRATIONS SUCH as amplifications, translocations, and nonrandom deletions are among important mechanisms underlying human oncogenesis. In retinoblastoma and Wilms' tumor, deletion of specific chromosomal regions was detected that resulted in homozygosity of particular alleles on chromosome 13 and 11, respectively (*1, 2*). The deletion of normal cellular sequences, which is thought to unmask recessive mutations, may not be limited to pediatric tumors (*3, 4*). In particular, restriction fragment length polymorphism (RFLP) analyses have demonstrated loss of genes on specific chromosomes in hereditary disorders and other adult malignancies (*5–8*).

A loss of one of the c-H-*ras*-1 (chromosome 11p) alleles was demonstrated in primary breast tumor DNAs from 27% of patients constitutionally heterozygous for this locus (*9*). Here we present analysis of additional genetic markers on chromosome 11 (five on 11p and one on 11q) and single markers on six other autosomes in breast tumors DNAs (Table 1) (*10–21*). In 11 of 56 patients (20%) who were heterozygous at multiple loci on chromosome 11 there was loss of heterozygosity at one or more loci.

DNA sequences of variable lengths on chromosome 11 were lost in these 11 breast cancer patients (Fig. 1 and Table 2). The tumor DNAs of patients 299 and 243 were reduced to homozygosity at the γ^A fraction of the β-globin locus. Patient 304 showed an allelic loss at the parathyroid hormone (PTH) locus in her tumor DNA, whereas a loss of heterozygosity had occurred at the calcitonin loci in patients 86 and 315. In addition to the loss of heterozygosity at the c-H-*ras*-1 and PTH loci, tumor DNA from patient 295 was also missing an allele at the catalase locus, suggesting that the entire short arm of chromosome 11 might have been lost. Patient 253 represented the only case in this study where loss of heterozygosity occurred on 11p markers (c-H-*ras*-1 γ^G, and γ^A) as well as at the *int*-2 locus on 11q in the tumor DNA. This suggests a possible loss of an entire chromosome 11 homolog in the tumor cells of this patient.

There was a simultaneous loss of heterozygosity at the c-H-*ras*-1 and γ-globin loci in patients who were constitutionally heterozygous at these loci (Table 2, patients 299, 253, 86, and 180). Three other patients (166, 295, and 304), who had an allelic loss at the c-H-*ras*-1 locus in their tumor DNAs, were homozygous for both γ^G and γ^A regions of the globin locus. In these cases of constitutional homozygosity the intensity of bands in tumor DNA was markedly less than that of the lymphocyte DNA from the same patient (Fig. 1a) suggesting a loss of allele at the γ-globin loci.

The data from three other patients (223, 180, and 408) are presented separately in Fig. 2 in an attempt to define the shortest region deleted from the tumor DNAs of breast cancer patients. A reduction to homozygosity had occurred at the PTH and calcitonin loci in the tumor DNA of patient 223. The presence of a reduced signal (20 to 40% of normal) of the 2.2-kb PTH and the 8.0-kb calcitonin alleles was probably due to contamination of the tumor biopsies with normal cells. This patient was constitutionally heterozygous at the γ^A region of the β-globin locus. However, a loss of heterozygosity was not observed at this locus. In addition, DNA from patient 223 was homo-

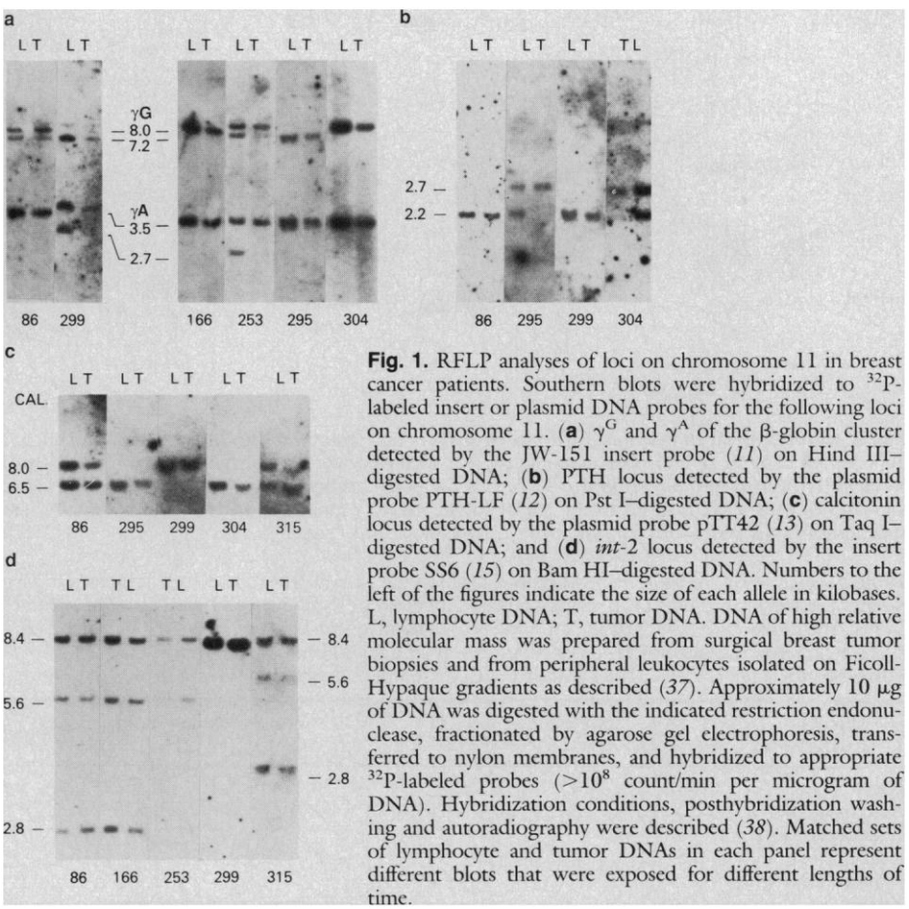

Fig. 1. RFLP analyses of loci on chromosome 11 in breast cancer patients. Southern blots were hybridized to ^{32}P-labeled insert or plasmid DNA probes for the following loci on chromosome 11. (**a**) γ^G and γ^A of the β-globin cluster detected by the JW-151 insert probe (*11*) on Hind III–digested DNA; (**b**) PTH locus detected by the plasmid probe PTH-LF (*12*) on Pst I–digested DNA; (**c**) calcitonin locus detected by the plasmid probe pTT42 (*13*) on Taq I–digested DNA; and (**d**) *int*-2 locus detected by the insert probe SS6 (*15*) on Bam HI–digested DNA. Numbers to the left of the figures indicate the size of each allele in kilobases. L, lymphocyte DNA; T, tumor DNA. DNA of high relative molecular mass was prepared from surgical breast tumor biopsies and from peripheral leukocytes isolated on Ficoll-Hypaque gradients as described (*37*). Approximately 10 μg of DNA was digested with the indicated restriction endonuclease, fractionated by agarose gel electrophoresis, transferred to nylon membranes, and hybridized to appropriate ^{32}P-labeled probes (>10^8 count/min per microgram of DNA). Hybridization conditions, posthybridization washing and autoradiography were described (*38*). Matched sets of lymphocyte and tumor DNAs in each panel represent different blots that were exposed for different lengths of time.

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zygous at the c-H-*ras*-1, catalase, and *int*-2 loci. The lack of demonstrable reduction in gene dosage between the lymphocyte and tumor DNAs at these loci (as measured by densitometry) suggests that sequence deletion is confined to the region between the β-globin and catalase loci of chromosome 11.

Tumor DNA from patient 180, which lacks one of the c-H-*ras*-1 alleles (*9*), also has lost one of the two alleles at the γ^G locus of the β-globin cluster (Fig. 2). However, heterozygosity at the PTH locus was maintained in the tumor DNA of this patient. Patient 408 was missing the c-H-*ras*-1 allele in the tumor DNA but maintained heterozygosity at the calcitonin locus. This patient was constitutionally homozygous at the γ-globin and PTH loci. Even so, the intensity of bands at the γ^G and γ^A loci was considerably less in the tumor DNA than in the lymphocyte DNA as was the case with patients 166, 295, and 304 (*22*).

The maintenance of heterozygosity at the calcitonin locus in the tumor DNA from patient 408 provides evidence that the critical region in breast tumors is different from the WGAR (Wilms' tumor, aniridia, genitourinary malformation, and mental retardation) locus (*23*). The hybridization profiles of patients 223 and 180 revealed the reciprocal nature of genotypes (loss of PTH and calcitonin alleles and maintenance of heterozygosity at the γ-globin locus in patient 223; loss of γ-globin alleles and maintenance of heterozygosity at the PTH locus in patient 180). Thus, the region between the γ-globin and PTH loci might be important in the genesis of breast tumors.

Our data demonstrate a loss of heterozygosity for multiple loci on chromosome 11 (Table 2 and Fig. 3). The deletions were of variable length with no common breakpoint at either end. Moreover, most of the deletions observed in the breast tumor DNAs include c-H-*ras*-1 and β-globin loci. Deletions in this region seem to be quite common in DNA from tumors of epithelial origin (*2, 5, 9, 24*). A recent study characterizing the deletions occurring in cases of hereditary persistence of fetal hemoglobin found that the region downstream from the β-globin gene was highly recombinogenic (*25*). Somatic deletions of c-H-*ras*-1 and γ^G and γ^A loci are frequently observed in Wilms' tumor patients (*2*). However, studies of patients having germline interstitial deletions of 11p13 and a predisposition to Wilms' tumor have shown that both of these loci are outside the Wilms' tumor locus (*26*). More recent studies have placed the WGAR locus between the FSHB (β subunit of follicle-stimulating hormone) and catalase genes (*23*). Thus, the allelic loss of the c-H-*ras*-1 and globin loci described in previous studies and presented here may be coincidental and possibly reflects the genetic instability of this region of chromosome 11. Consistent with this rationale is the presence of an inheritable fragile site located at 11p13 (*27*). It is conceivable that breakage at this fragile site may result in overlapping deletions of one or more loci on chromosome 11 for different malignancies.

The question of the frequency of deletions

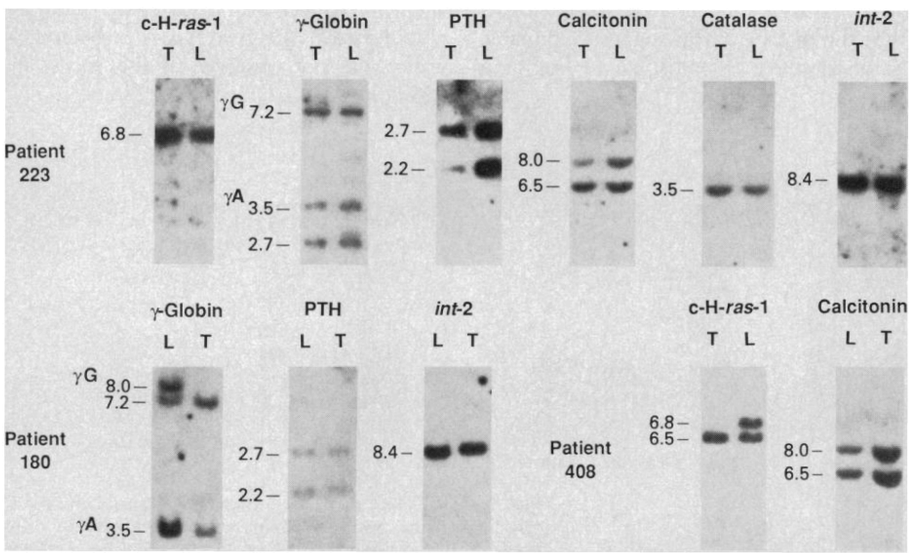

Fig. 2. Constitutional and tumor genotypes of markers on chromosome 11 in patients 223, 180, and 408. An insert probe of c-H-*ras*-1 plasmid (*10*) was used to detect the polymorphism of the c-H-*ras*-1 locus (*39*). The plasmid probe pSSI (*14*) detects the polymorphism of the catalase gene. The size in kilobases of each allele is given to the left of the figure. L, lymphocyte DNA; T, tumor DNA. The isolation of DNA and Southern blot analyses are as described (Fig. 1).

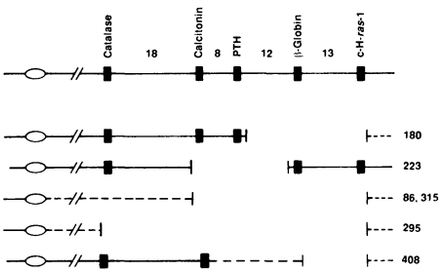

Fig. 3. A schematic representation of the deletions on the short arm of chromosome 11 in breast cancer patients. The order and distance between the markers on chromosome 11p is according to published recombination percentages (*40*). Numbers between genetic loci refer to recombination percentages or distances in centimorgans. Vertical lines mark the beginning or end of the deletion at a heterozygous locus. Broken lines indicate that the markers were either not informative (catalase in patients 86 and 315; γ-globin and PTH in patient 408) or not done (covering the region between catalase and centromere and the region between c-H-*ras*-1 and telomere) and therefore, the deletion could theoretically extend beyond these loci. Patient 223 was homozygous for the c-H-*ras*-1 and catalase loci, but the densitometric scanning of the autoradiogram (Table 2) suggests that the deletion region does not include these loci in this patient.

Table 1. Analysis of polymorphic markers on chromosome 11 and other autosomes in breast cancer patients. The loci on chromosome 11 were detected by means of recombinant DNA probes (shown in parentheses) for the human c-H-*ras*-1 (*9, 10*), γ-globin (*11*), PTH (*12*), calcitonin (*13*), catalase (*14*), and *int*-2 (*15*). The probes pgHS7-2.7 or somatostatin (*16*), c-*myb* (*17*), *met* H (*18*), p9A7 (*19*), c-*erb*-A2 (*20*), and v-*sis* (*21*) were used for chromosomes 3, 6, 7, 13, 17, and 22, respectively. Bam HI–digested DNA was used for c-H-*ras*-1, SS6, pgHS7-2.7, and c-*erb*A-2; Hind III was used for JW-151, p9A7, and v-*sis*; Pst I was used for PTH-LF; Taq 1 was used for pTT42, pSSI, and *met* H; and Eco RI was used for c-*myb*.

Patients analyzed	c-H-*ras*-1 (c-H-ras-1)	γ-globin (JW-151)	PTH (PTH-LF)	Calcitonin (pTT42)	Catalase (pSSI)	*int*-2 (SS6)	pgHS7-2.7	c-*myb*	*met* H	p9A7	c-*erb*-A2	v-*sis*
Total	104	47	45	26	20	104	60	49	20	40	21	30
Heterozygous	51	33	20	12	3	53	9	27	8	18	10	12
Heterozygous (allelic loss in tumor DNA)	15	5	3	3	1	1	0	2	0	0	2	0

on other autosomes was also examined in breast cancer patients. A loss of heterozygosity occurred in 2 out of 27 informative patients at the c-*myb* locus and 2 out of 10 informative patients at the c-*erb*A-2 locus (Table 1). One of the patients (253) with a loss of a c-*myb* allele and one of the patients (180) with a loss of a c-*erb*A-2 allele had deletions at multiple loci on chromosome 11 (Table 2). The deletion of a c-*myb* allele in breast cancer patients has been reported (*24*).

The loss of heterozygosity on specific chromosomes in embryonal malignancies has been attributed to different mitotic mechanisms such as nondisjunction (alone or followed by reduplication), mitotic recombination, or gene conversion (*1, 28*). However, in breast tumors, hybridization signals of most of the cases heterozygous for markers on 11p are consistent with simple deletion of sequences on the short arm of chromosome 11 (Figs. 1 and 2). The frequency of deletions on 11p in breast carcinomas points to the existence of regulatory sequences that may be important in the genesis of breast tumors.

Evidence for the existence of normal tumor suppressor genes on various chromosomes has been provided by somatic cell hybrid experiments (*29–31*). Chromosome 11, especially sequences on its short arm, are implicated in the suppression of tumorigenicity of Hela cells (*29*). Since this cell line is also of epithelial origin it may be more than coincidence that deletion of sequences from the short arm of chromosome 11 was detected in different neoplasms of epithelial origin including Wilms' tumor of embryonic kidney (*2*), hepatoblastoma (*32*), hepatocellular carcinoma (*33*), transitional cell carcinoma of bladder (*5*), and breast cancer.

The deletion of sequences on chromosome 11 was observed in approximately 20% of the tumors from patients whose lymphocyte DNAs were informative for one or more chromosome 11 markers. In the previous study (*9*) a significant association was reported between the loss of a c-H-*ras*-1 allele and several clinical parameters including estrogen- and progesterone receptor–negative tumors, histopathological grade III tumors, and patients who developed distal metastasis. When tumors with allelic losses at other loci on chromosome 11 are included a stronger association is observed between deletions occurring at 11p and progesterone receptor–negative tumors (72%, $P < 0.002$) and histopathological grade III tumors (71%, $P < 0.006$). The other associations were not significantly changed (Table 3).

The results presented here also highlight the existence of a putative locus between the β-globin and PTH loci which, when deleted, might contribute to the genesis of breast cancer. However, due to the variable and sometimes low frequency of heterozygosity of the genes studied in this analysis, an expanded study of additional breast tumor DNAs with other polymorphic loci on 11p will be necessary to confirm this conclusion. In addition, genetic alterations involving genes on other chromosomes must be considered. Although frequent oncogenic point mutation of c-H-*ras*-1 at amino acid 12 was not detected in breast tumors (*9*), amplification of c-*myc* (*34*) and *neu*/c-*erb*B-2 (*35, 36*) has been found in several primary breast tumors. Future efforts to characterize the region between the β-globin and PTH loci on the short arm of chromosome 11 as well as to investigate the role of other proto-oncogenes should help in deciphering the complex events occurring individually or in concert during the evolution of breast neoplasia.

Table 2. Loss of constitutional heterozygosity of loci on chromosome 11 in tumor DNAs of breast cancer patients. Allelic losses occurring in patients 223 and 408 were important in characterizing the boundaries of shortest deletion region (see text). Therefore, a comparative scanning densitometry of the lymphocyte and tumor DNAs from patient 223 was carried out at homozygous loci (c-H-*ras*-1, catalase, and *int*-2). The results supported the presence of both alleles at these loci. Similar dosage analysis could not be performed at the homozygous loci (γ-globin and PTH) in case of patient 408 because the autoradiograms were not of sufficiently high quality; a and b refer to larger and smaller allelic fragments, respectively; - indicates loss of an allele; ND indicates not done because of the limited availability of tumor DNA.

Patients	c-H-*ras*-1	γ^G	γ^A	PTH	Calcitonin	Catalase	*int*-2
299	a/-	b/b	a/-	b/b	a/a	a/a	a/a
243	ND	a/a	a/-	ND	ND	ND	ND
166	a/-	a/a	a/a	a/a	ND	ND	a/b
304	a/a	a/a	a/a	a/-	b/b	a/a	ND
86	a/-	a/-	a/a	b/b	-/b	a/a	a/b
315	a/-	ND	ND	a/a	-/b	a/a	a/b
295	-/b	b/b	a/a	a/-	b/b	a/-	a/a
253	a/a	a/-	a/-	ND	ND	ND	a/-
223	a/a	b/b	a/b	a/-	-/b	a/a	a/a
180	a/-	-/b	a/a	a/b	ND	ND	a/a
408	a/-	b/b	a/a	b/b	a/b	ND	ND

Table 3. Correlation between chromosome 11p deletion and clinico-histopathological parameters.

Parameter*	Total tumor population [*n* (%)]	Tumors with deletions on chromosome 11 [*n* (%)]	Tumors without deletions [*n* (%)]	χ^2 analysis
I	10 (14)	0 (0)	10 (18)	
II	34 (47)	5 (29)	29 (53)	
III	28 (39)	12 (71)	16 (29)	$P < 0.006$
ER^+	55 (66)	8 (44)	47 (72)	$P < 0.025$
ER^-	28 (34)	10 (56)	18 (28)	$P < 0.0025$
PR^+	49 (59)	5 (28)	44 (68)	
PR^-	34 (44)	13 (72)	21 (32)	
M^-	66 (79)	11 (61)	55 (83)	$P < 0.05$
M^+	18 (21)	7 (39)	11 (17)	

*I, II, and III represent histopathologic grades; ER, estrogen receptor; PR, progresterone receptor; M^- and M^+, the absence or presence (respectively) of distal metastasis or local reoccurrence.

REFERENCES AND NOTES

1. W. K. Cavenee *et al.*, *Nature (London)* **305**, 779 (1983); W. F. Benedict *et al.*, *Science* **219**, 973 (1983); T. P. Dryja *et al.*, *N. Engl. J. Med.* **310**, 550 (1984).
2. A. Koufos *et al.*, *Nature (London)* **309**, 170 (1984); S. H. Orkin, D. S. Goldman, S. E. Sallan, *ibid.*, p. 172; A. E. Reeve *et al.*, *ibid.*, p. 174; E. R. Fearon, B. Vogelstein, A. P. Feinberg, *ibid.*, p. 176.
3. J. Whang-Peng *et al.*, *Science* **215**, 181 (1982); J. J. Yunis, *ibid.* **221**, 227 (1983).
4. R. Abe, A. A. Sandberg, *Cancer Genet. Cytogenet.* **13**, 121 (1984); A. Reichman, P. Martin, B. Levin, *ibid.*, p. 121; M. B. Atkin and M. C. Baker, *Hum. Genet.* **70**, 359 (1985); N. Maeski, Y. Kaneko, M. Sakurai, *Jpn. J. Cancer Res. (GANN)* **77**, 334 (1986).
5. E. R. Fearon, A. P. Feinberg, S. H. Hamilton, B. Vogelstein, *Nature (London)* **318**, 377 (1985).
6. L. M. Kunkel, *ibid.* **322**, 73 (1986).
7. B. R. Seizinger *et al.*, *ibid.*, p. 644.
8. S. Mukai and T. P. Dryja, *Cancer Genet. Cytogenet.* **22**, 45 (1986).
9. C. Theillet *et al.*, *Cancer Res.* **46**, 4776 (1986).
10. E. H. Chang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4848 (1982).
11. S. E. Antonarakis *et al.*, *ibid.*, p. 137.
12. S. E. Antonarakis *et al.*, *ibid.* **80**, 6615 (1983). The probe does not recognize the 2.2-kb common band (P. O'Connell, personal communication).
13. J. W. M. Hoeppener *et al.*, *Hum. Genet.* **66**, 309 (1984).

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).
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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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