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Activation of related transforming genes in mouse and human mammary carcinomas

(mouse mammary tumor virus/chemical carcinogenesis/human tumors/transfection)

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ABSTRACT **High molecular weight DNAs of five tumors induced by mouse mammary tumor virus (MMTV), two mouse mammary tumors induced by a chemical carcinogen, and one human mammary tumor cell line (MCF-7) were assayed for the presence of transmissible activated transforming genes by transfection of NIH 3T3 mouse cells. DNAs of all five MMTV-induced tumors, one chemical carcinogen-induced tumor, and the human tumor cell line induced transformation with high efficiencies (≈0.2 transformant per μg of DNA). NIH cells transformed by DNAs of MMTV-induced tumors did not contain exogenous MMTV DNA sequences, indicating that MMTV-induced mammary carcinomas contained activated cellular transforming genes that were not linked to viral DNA. The transforming activities of DNAs of all five MMTV-induced tumors, the chemical carcinogen-induced mouse tumor, and the human tumor cell line were inactivated by digestion with the restriction endonucleases *Pvu* II and *Sac* I, but not by *Bam*HI, *Eco*RI, *Hin*dIII, *Kpn* I, or *Xho* I. These results indicate that the same or closely related transforming genes were activated in six different mouse mammary carcinomas, induced by either MMTV or a chemical carcinogen, and in a human mammary carcinoma cell line.**

Cellular genes capable of inducing oncogenic transformation have recently been identified by transfection of DNAs of both normal and neoplastic cells (1–5). The low efficiency of transformation (≈0.003 transformant per μg of DNA) induced by DNA fragments of normal cells appears to represent activation of normal cell genes with potential transforming activity, because cells transformed by normal cell DNA fragments contain activated transforming genes that can be transmitted at high efficiencies (0.1–1 transformant per μg of DNA) in secondary transfection assays (1). Activated transforming genes that can be efficiently transmitted by transfection with high molecular weight DNAs have also been found in chemically transformed mouse fibroblasts (2), lymphomas and a nephroblastoma induced by avian lymphoid leukosis viruses (3), and human bladder carcinomas (4, 5). The transforming genes detected by transfection of DNAs of tumors induced by lymphoid leukosis viruses, a class of weakly oncogenic retroviruses that lack viral transforming genes, are not linked to viral DNA sequences, suggesting that oncogenesis by these viruses involves indirect activation of cellular transforming genes (3). These findings thus suggest that transformation by a variety of carcinogenic agents can involve dominant mutations or gene rearrangements resulting in the activation of cellular transforming genes, which are then detectable by transfection.

In the present study, we have investigated the transforming activities of DNAs of mouse mammary carcinomas, induced by either mouse mammary tumor virus (MMTV) or dimethylbenzanthracene (DMBA), and of a human mammary carcinoma cell line. MMTV is a weakly oncogenic retrovirus that, like the avian lymphoid leukosis viruses, does not appear to contain viral transforming genes. Endogenous MMTV genomes are normally present in DNAs of most mice, but oncogenesis by MMTV appears to be dependent upon exogenous virus infection (6–10). In C3H mice, mammary tumors occur with high frequency as a consequence of exogenous infection by milk-transmitted MMTV (6, 7). Other strains of mice, such as BALB/c, do not transmit infectious exogenous MMTV and have a much lower incidence of spontaneous mammary carcinomas (6, 7).

High molecular weight DNAs of five MMTV-induced C3H mammary carcinomas, one DMBA-induced BALB/c mammary carcinoma, and the human mammary carcinoma cell line MCF-7 induced efficient transformation of NIH 3T3 cells, indicating that these mouse and human mammary carcinomas contained activated transforming genes. As in the case of neoplasms induced by avian lymphoid leukosis virus (3), the transforming genes of MMTV-induced tumors were not linked to viral DNA sequences. Analysis of the transforming activities of these tumor DNAs by digestion with restriction endonucleases indicated that the same or closely related transforming genes were activated in each of the six mouse mammary carcinomas and in the human mammary carcinoma cell line.

MATERIALS AND METHODS

Tumor and Cell Lines. MMTV-induced mammary tumors were obtained from C3H/HeSFCI *nu*/+ retired breeder females. The SFCI colony was established 3 years ago with C3H/HeN *nu*/+ females and C3H/HeN *nu*/*nu* males obtained from K. Hansen (National Cancer Institute). Tumor incidence is 100% in multiparous females and average age at tumor onset is 7.5 months. Normal mammary glands were obtained from tumor-free multiparous females 2 weeks after lactation. Livers were obtained from C3H/HeSFCI *nu*/+ males at 6 weeks of age. Transplantable DMBA-induced mammary tumors of the BALB/cCRGL strain, designated D1-DMBA-3 and D1-DMBA-4 (11), were a gift from D. Lopez (University of Miami). Livers were obtained from young BALB/cCRGL females. The human breast carcinoma cell line, MCF-7 (12), was obtained from L. B. Chen (Sidney Farber Cancer Institute). Normal human embryo lung fibroblasts (IMR90 cells) (13) were obtained from J. Rheinwald (Sidney Farber Cancer Institute).

Transfection Assays. Tumor tissues, livers, and normal mammary glands were rapidly removed, minced, and homogenized in a Dounce homogenizer. DNAs were extracted from tumor tissues, cell suspensions, and mammary glands as described (14). Livers were processed similarly, except that the initial Pronase digestion was omitted and the sodium dodecyl

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Abbreviations: MMTV, mouse mammary tumor virus; DMBA, dimethylbenzanthracene; LTR, long terminal redundancy; kb, kilobase(s).

sulfate concentration was 1.5%. The molecular weights of all DNAs were greater than 30 million as estimated by electrophoresis in agarose gels.

The transforming activities of high molecular weight DNAs were assayed by transfection of NIH 3T3 mouse cells as described (15, 16), except that dexamethasone (0.5 μM) was included in all culture media after DNA treatment. This was found to increase focus formation approximately 2-fold and to decrease the time required to detect foci by 2–3 days. The effect of dexamethasone was not specific for mammary tumor DNAs, because similar stimulations were observed in assays of DNAs of NIH cells transformed by Rous sarcoma virus DNA, lymphoid leukosis virus-induced tumor DNAs, and normal cell DNA fragments. In most experiments, foci of transformed cells were counted 10–14 days after exposure to DNA. In some experiments, DNA-treated cultures were transferred into soft agarose-containing medium 7 days after exposure to DNA, and colonies of transformed cells were counted after 3–4 weeks of further incubation (1).

Restriction Endonuclease Digestions, Agarose Gel Electrophoresis, and Hybridization. Procedures for digestion of DNAs with restriction endonucleases, electrophoresis in agarose gels, transfer to nitrocellulose filters, and hybridization were as described (14). [^{32}P]DNA probes were prepared by nick-translation (17) of plasmids containing MMTV DNA (18) sequences, generously provided by G. Hager (National Cancer Institute).

RESULTS

Transforming Activity of Mammary Carcinoma DNAs. High molecular weight DNAs of five MMTV-induced tumors of C3H mice, two DMBA-induced tumors of BALB/c mice, and the human mammary carcinoma cell line MCF-7 were assayed for transforming activity by transfection of NIH 3T3 cells. Efficient transformation (0.1–0.4 foci per μg of DNA) was induced by DNAs of all five MMTV-induced tumors, one of the DMBA-induced tumors (D1-DMBA-4), and the human MCF-7 cell line (Table 1). Similar transformation efficiencies were obtained when DNAs of these tumors were assayed for colony formation in soft agarose (data not shown). In contrast, DNA of one of the DMBA-induced tumors (D1-DMBA-3) and control DNAs extracted from normal lactating mammary glands of MMTV-infected C3H mice, livers of BALB/c mice, and normal human embryo fibroblasts lacked significant transforming activity (Table 1). Foci of NIH cells transformed by all of the mouse mammary carcinoma DNAs and by DNA of the MCF-7 cell line were indistinguishable from each other and contained highly vacuolated cells that differed from NIH cells transformed by normal cell DNAs, lymphoid leukosis virus-induced tumor DNAs, or human bladder carcinoma DNAs (1, 3, 4).

Table 1. Transforming activity of mammary carcinoma DNAs

Donor DNA	Total foci/ total recipient cultures	Foci/μg DNA
MMTV-induced C3H tumors		
MMTV-Tu1	31/8	0.19
MMTV-Tu2	46/15	0.15
MMTV-Tu3	41/10	0.21
MMTV-Tu4	74/10	0.37
MMTV-Tu5	46/10	0.23
DMBA-induced BALB/c tumors		
D1-DMBA-3	3/40	0.004
D1-DMBA-4	66/30	0.11
Human tumor cell line		
MCF-7	49/14	0.17
Controls		
C3H mammary glands (4)	3/40	0.004
BALB/c livers (2)	0/20	<0.003
Normal HEF	1/20	0.003
Salmon sperm	1/46	0.001

NIH 3T3 cells were exposed to 20 μg of DNA per recipient culture and foci were counted 10–14 days after transfection. Data obtained with DNAs of four sets of C3H mammary glands and two BALB/c livers are pooled. HEF, human embryo fibroblasts.

Individual foci of transformed NIH cells were grown to mass cultures and used as donors of DNA in secondary transfection assays. DNAs of all foci of NIH cells transformed by mammary carcinoma DNAs induced transformation with efficiencies comparable to the initial efficiency of transformation by the tumor DNAs (Table 2). The morphologies of NIH cells transformed in secondary transfection assays were indistinguishable from the morphologies of the transformed NIH cells used as DNA donors. Because DNAs of spontaneously transformed NIH cells lack transforming activity (1, 3, 4, 15), these results indicate that NIH cells transformed by mammary carcinoma DNAs contain transmissible activated transforming genes derived from the tumor DNAs.

Tumorigenicity of Transformed NIH Cells. NIH 3T3 cells, NIH cells transformed by MCF-7 DNA, and NIH cells transformed by MMTV-Tu2 DNA were injected intraperitoneally into 8-week-old BALB/c mice. All mice injected with 1×10^6 NIH cells transformed by either MCF-7 DNA (four mice) or MMTV-Tu2 DNA (three mice) developed multiple focal tumors throughout the peritoneal cavity within 7–10 days. The tumors grew progressively and killed the host within 3 weeks. In contrast, no tumors developed in mice injected with 1×10^7 normal NIH 3T3 cells. The lack of tumor rejection may reflect partial histocompatability matching between NIH 3T3 cells and BALB/c mice, bypass of normal immune rejection by the intraperitoneal route of inoculation, or both. Histologic examination indicated that the tumors were undifferentiated fibrosarcomas.

Absence of Exogenous MMTV DNA in NIH Cells Transformed by DNAs of MMTV-Induced Tumors. NIH cells transformed by DNAs of the five MMTV-induced tumors, the D1-DMBA-4 tumor, and the MCF-7 cell line did not produce virus particles detectable by assays of culture fluids for focus formation on NIH 3T3 cells, MMTV sedimentable DNA polymerase activity (19), or murine leukemia virus sedimentable DNA polymerase activity (15). To determine whether transforming genes of MMTV-induced tumors were linked to exogenous MMTV sequences, DNAs of NIH cells transformed by these tumor DNAs were analyzed by digestion with restriction endonucleases and Southern blot hybridization.

Table 2. Transformation by DNAs of NIH cells transformed by mammary carcinoma DNAs

Donor DNA	Total foci*	Foci/μg DNA
NIH(MMTV-Tu1)	14, 17, 14, 7, 10, 10	0.10
NIH(MMTV-Tu2)	45, 38, 40, 64	0.39
NIH(MMTV-Tu3)	36, 23, 15	0.20
NIH(MMTV-Tu4)	18, 14, 25	0.16
NIH(MMTV-Tu5)	23, 30	0.22
NIH(D1-DMBA-4)	14, 16, 9, 18, 13, 23	0.13
NIH(MCF-7)	31, 44, 34, 21	0.27

DNAs of two to six individual foci of NIH cells transformed by each of the designated mammary carcinoma DNAs were assayed by transfection of NIH 3T3 cells. Salmon sperm DNA, included as a negative control in all assays, had a transforming activity of 0.003 foci per μg of DNA.

* Each number represents the total foci obtained from six recipient cultures exposed to DNA from each individual focus of transformed NIH cells at 20 μg per culture.

Fig. 1 illustrates the results of experiments in which DNAs were digested with *Eco*RI, which cleaves at one internal site in MMTV DNA (9), and hybridized to [^{32}P]DNA of a plasmid containing the 1.3-kilobase (kb) long terminal redundancy (LTR) of MMTV DNA cloned in plasmid pBR322 (18). DNAs of each of the five MMTV-induced C3H mammary tumors yielded MMTV DNA-containing *Eco*RI fragments that were not present in C3H liver DNA and thus represented integration sites of exogenous MMTV proviruses (Fig. 1). The reduced intensity of hybridization to some exogenous fragments compared to endogenous fragments in the tumor DNAs is consistent with each exogenous provirus being represented only once per cell genome and with the possibility that the tumors contain some normal tissue. In contrast to DNAs of the MMTV-induced tumors, DNAs of NIH cells transformed by DNAs of these tumors yielded only the endogenous MMTV DNA-containing *Eco*RI fragments that were present in DNA of normal NIH 3T3 cells (Fig. 1). Similar results were obtained when DNAs were digested with *Pst* I, which cleaves at multiple sites in MMTV DNA (9), and when blots were hybridized with [^{32}P]DNA prepared from a plasmid containing approximately the 3′ one-third of the MMTV genome (G. Hager, personal communication) (data not shown). NIH cells transformed by DNAs of MMTV-induced tumors thus did not appear to contain exogenous MMTV sequences, indicating that the transforming genes of these tumors were not linked to viral DNA.

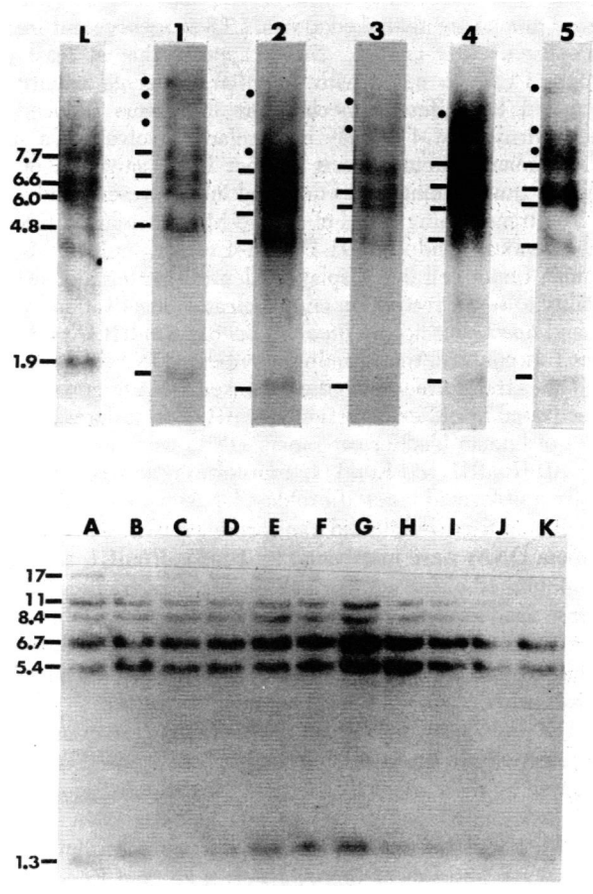

FIG. 1. Analysis of MMTV DNA in tumors and transformed NIH cells. DNAs (10 μg) were digested with *Eco*RI and analyzed by blot hybridization with [^{32}P]DNA of a plasmid containing the MMTV LTR. (*Upper*) DNAs of C3H liver (L) and of MMTV-induced C3H mammary tumors 1, 2, 3, 4, and 5. Endogenous MMTV DNA-containing fragments are indicated by bars and their lengths are shown in kb. Tumor-specific MMTV DNA-containing fragments are indicated by dots. (*Lower*) DNAs of normal NIH 3T3 cells (G) and of two lines of NIH cells transformed by DNAs of MMTV-Tu1 (A, B), MMTV-Tu2 (C, D), MMTV-Tu3 (E, F), MMTV-Tu4 (H, I), and MMTV-Tu5 (J, K). The lengths of endogenous MMTV DNA-containing fragments are shown in kb.

Characterization of Mammary Carcinoma Transforming Genes by Digestion with Restriction Endonucleases. The transforming genes of MMTV-induced, DMBA-induced, and MCF-7 mammary carcinomas were further characterized by investigating the sensitivity of DNAs of both mammary carcinomas and NIH cells transformed by mammary carcinoma DNAs to digestion with seven different restriction endonucleases (Table 3). The transforming activities of all DNAs were inactivated by digestion with *Pvu* II and *Sac* I, but not by digestion with *Bam*HI, *Eco*RI, *Hin*dIII, *Kpn* I, and *Xho* I. This pattern of sensitivity to restriction endonuclease digestion differs from that of the transforming activities of DNAs of NIH cells transformed by normal cell DNA fragments (1), chemically transformed mouse fibroblasts (20), human bladder carcinomas (4), or avian lymphoid leukosis virus-induced lymphomas (unpublished observations). These results thus indicate that the same or closely related transforming genes were activated in all five MMTV-induced tumors, the D1-DMBA-4 tumor, and the human MCF-7 cell line.

*Eco*RI-digested DNA of NIH cells transformed by MCF-7 DNA was fractionated by sucrose gradient sedimentation and individual fractions were assayed for transforming activity (Fig. 2). Transforming activity sedimented as a single peak with a molecular weight corresponding to approximately 9 kb. The specific transforming activity of the peak fractions (10 and 11) corresponded to 1–1.2 transformants per μg of DNA, representing a 5-fold increase in specific activity compared to unfractionated DNA. The transforming activity of *Eco*RI-digested

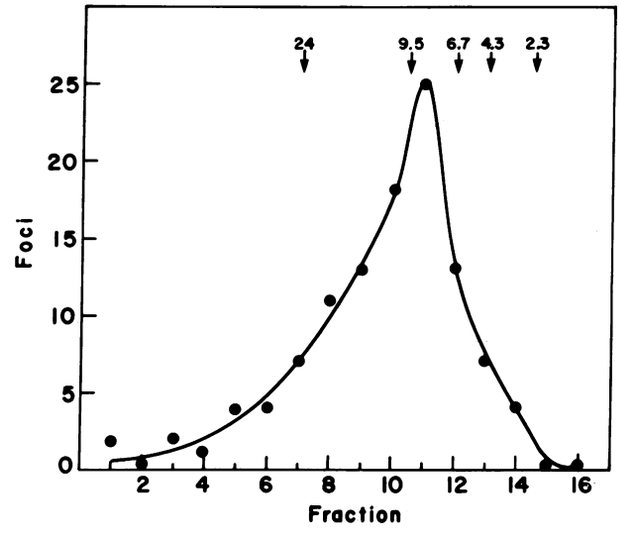

FIG. 2. Sucrose gradient fractionation of *Eco*RI-digested DNA. *Eco*RI-digested DNA (1 mg) of NIH cells transformed by MCF-7 DNA was centrifuged through three 30-ml 10–40% sucrose gradients at 25,000 rpm for 17 hr in a Beckman SW 27 rotor. Sixteen fractions were collected from each of the three gradients, homologous fractions were pooled, and DNAs were concentrated by ethanol precipitation. Aliquots were used to determine DNA concentrations by absorbance and molecular weights by electrophoresis in agarose gels. One-third of the DNA of each fraction was mixed with 60 μg of salmon sperm carrier DNA, and transforming activity was assayed by transfection of four recipient cultures of NIH 3T3 cells. Total foci obtained in this assay are shown for each fraction. Molecular sizes (kb) are indicated by arrows and correspond to the cellular DNA fraction that comigrated in agarose gels with marker fragments of *Hin*dIII-digested λ DNA.

Table 3. Digestion of transforming DNAs with restriction endonucleases

Donor DNA	Foci/μg DNA							
	Undigested	*Bam*HI	*Eco*RI	*Hin*dIII	*Kpn* I	*Pvu* II	*Sac* I	*Xho* I
MMTV-Tu1	0.17	0.19	0.14	0.14	0.25	<0.01	0.01	0.10
MMTV-Tu2	0.23	0.39	0.19	0.21	0.29	<0.01	0.01	0.14
NIH(MMTV-Tu2)	0.24	ND	0.13	0.28	0.30	<0.01	0.01	0.19
NIH(MMTV-Tu3)	0.20	0.28	0.19	0.19	0.31	0.01	<0.01	0.15
MMTV-Tu4	0.23	0.30	0.17	0.28	0.21	<0.01	<0.01	0.14
NIH(MMTV-Tu5)	0.34	0.54	0.30	0.33	0.45	<0.01	<0.01	0.48
D1-DMBA-4	0.18	0.25	0.21	0.14	0.26	<0.01	<0.01	0.09
NIH(MCF-7)c11	0.26	0.38	0.22	0.22	0.21	<0.01	0.02	0.12
NIH(MCF-7)c12	0.30	0.44	0.26	0.32	0.29	<0.01	0.02	0.21

DNAs (80–120 μg) of either mammary carcinomas or of NIH cells transformed by mammary carcinoma DNAs were digested to completion with the indicated restriction endonucleases. Bacteriophage λ DNA (2 μg) was included in a separate aliquot of each reaction mixture that was incubated in parallel and analyzed by electrophoresis in agarose gels to monitor the specificity and extent of digestion. Transforming activities of undigested and digested DNAs were assayed by focus formation. NIH(MCF-7)c11 and -c12 cells are two different lines of NIH cells transformed by MCF-7 DNA. ND, not done.

DNA thus appeared to be associated with a single size class of DNA fragments.

DISCUSSION

The high transforming activities of DNAs of five MMTV-induced mouse mammary carcinomas, one DMBA-induced mouse mammary carcinoma, and a human mammary carcinoma cell line suggest that oncogenesis in these tumors involved dominant mutations or gene rearrangements resulting in activation of cellular transforming genes to a state in which they were efficiently transmissible by transfection. In contrast, DNA of one DMBA-induced mouse tumor, D1-DMBA-3, like the DNAs of the majority of chemically transformed mouse fibroblasts (2) and human tumors (4), lacked significant transforming activity. Oncogenesis in this tumor may have involved epigenetic changes or genetic changes, such as recessive mutations, which would not be transmissible by transfection. It is of interest that the two chemical carcinogen-induced tumors resulted from DMBA treatment of the same hormonally induced preneoplastic nodule outgrowth (11). The observation that DNA of D1-DMBA-4 induced efficient transformation, whereas DNA of D1-DMBA-3 lacked transforming activity, therefore suggests that transformation may have occurred by different mechanisms in two tumors derived from the same preneoplastic cell population.

NIH cells transformed by DNAs of MMTV-induced tumors did not contain exogenous MMTV DNA sequences. This indicates that the transforming genes of these tumors were not linked to viral DNA and suggests that oncogenesis by MMTV, as by avian lymphoid leukosis viruses (3), involves indirect activation of cellular transforming genes. Because tumorigenesis by both lymphoid leukosis viruses and MMTV requires a long latent period and involves several preneoplastic and neoplastic stages, it is possible that the viruses act at a stage of tumor development preceding or complementing the activation of transforming genes detected by transfection. Recent studies of Hayward *et al.* (21) have revealed that lymphoid leukosis virus-induced tumors contain exogenous viral LTR sequences integrated in the vicinity of the chicken gene (c-*myc*) homologous to the presumed transforming gene of a highly oncogenic acute leukemia virus. Integration of viral LTR sequences, which contain the viral transcriptional promoter, apparently results in increased expression of c-*myc*, implicating activation of this gene as a direct consequence of virus infection in the neoplastic process (21). Because the activated cellular transforming genes detected by transfection of DNAs of lymphoid leukosis virus-induced tumors are not linked to viral LTR sequences and are not homologous to c-*myc* (3, 22), it appears that at least two different cellular genes with potential oncogenic activity are activated by different mechanisms in tumors induced by these viruses. MMTV may be similarly involved in a stage of mammary carcinogenesis distinct from activation of the cellular transforming genes detected in the present study.

The transforming genes of all five MMTV-induced tumors, the chemically induced D1-DMBA-4 tumor, and the MCF-7 human tumor cell line displayed identical patterns of susceptibility to seven restriction endonucleases: inactivation by *Pvu* II and *Sac* I, but not by *Bam*HI, *Eco*RI, *Hin*dIII, *Kpn* I, and *Xho* I. In contrast, transforming activities of DNAs of three NIH cell lines transformed by normal chicken DNA fragments were inactivated by either *Bam*HI or *Hin*dIII (1); transforming activities of human bladder carcinoma DNAs were inactivated by *Eco*RI, *Hin*dIII, and *Kpn* I (4); transforming activities of chemically transformed mouse fibroblast DNAs were inactivated by *Eco*RI and *Hin*dIII (20); and transforming activities of avian lymphoma DNAs were inactivated by *Eco*RI, *Hin*dIII, and *Xho* I (unpublished observations). The results of restriction endonuclease analysis of the seven mammary carcinoma DNAs therefore indicate that the transforming genes activated in these tumors are structurally similar to each other but distinct from previously described cellular transforming genes.

The probability of different genes yielding the same pattern of sensitivity to the restriction endonucleases used can be estimated by the Poisson distribution from the average cleavage frequency of these enzymes in total cellular DNA. *Bam*HI, *Eco*RI, *Hin*dIII, *Pvu* II, *Sac* I, and *Xho* I cleave cellular DNAs at average intervals of approximately 4 kb, and *Kpn* I cleaves at average intervals of approximately 15 kb. Assuming a gene size of 4 kb, the probability of inactivation by *Bam*HI, *Eco*RI, *Hin*dIII, *Pvu* II, *Sac* I, or *Xho* I is 0.63, and the probability of inactivation by *Kpn* I is approximately 0.2. The probability of two different genes displaying the pattern observed for the mammary carcinoma DNAs is therefore $(0.37)^4(0.63)^2(0.8) = 0.006$. The probability of all seven tumor DNAs displaying this pattern by chance is $(0.006)^6 = 4 \times 10^{-14}$. The maximal likelihood of two genes displaying the observed pattern by chance would occur if the gene size was 1.6 kb. In this case, the probability of all seven tumor DNAs displaying the pattern is ap-

proximately 10^{-10}. It thus appears that the same or closely related transforming genes were activated in all seven mammary carcinomas.

To determine whether the expected evolutionary divergence between human and mouse genes was inconsistent with the identical pattern of sensitivity to seven restriction endonucleases observed for human and mouse mammary carcinoma DNAs, computer analysis of DNA sequences was used to compare the sensitivity of human and mouse β-globin (23, 24) and κ constant region (25) genes to cleavage by 18 six-base-recognition restriction endonucleases. In both gene pairs, 14 of these enzymes yielded the same pattern of cleavage sensitivity for the human and mouse gene homologs. It is, therefore, not extraordinary to obtain an identical pattern of sensitivity to seven enzymes in homologous genes activated in human and mouse mammary carcinomas.

Activation of related transforming genes in mouse mammary carcinomas induced by both viral and chemical carcinogens and in a cell line derived from a spontaneous human mammary carcinoma suggests the possibility that specific transforming genes are activated in neoplasms of particular tissue types. Recent studies of Shilo and Weinberg (20), indicating that the transforming genes activated in four chemically transformed lines of mouse fibroblasts were related to each other, are also consistent with this notion. One hypothesis suggested by these observations is that, in some differentiated cell types, only one or a few of the total number of cellular genes with potential transforming activity are susceptible targets for mutations or DNA rearrangements resulting in transforming gene activation. Alternatively, the total set of genes with potential transforming activity might be susceptible targets for activation in differentiated cells, but the state of differentiation of some cell types might render the cells phenotypically sensitive to the effects of only one or a few of these genes. In either case, it appears reasonable to speculate that genes involved in transformation of particular differentiated cell types might be normally involved in growth control of those cells, but not in cells of other differentiation pathways. Analysis of the normal functions of cellular transforming genes, and of the molecular events involved in transforming gene activation, may permit experimental evaluation of these hypotheses.

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