MOLECULAR AND CELLULAR BIOLOGY, Feb. 1989, p. 854–859
0270-7306/89/020854-06$02.00/0

Vol. 9, No. 2

Transgenic Mice Carrying the Mouse Mammary Tumor Virus *ras* Fusion Gene: Distinct Effects in Various Tissues

PATRICK J. TREMBLAY,[1] FRANÇOIS POTHIER,[1] TRANG HOANG,[2] GILLES TREMBLAY,[3] SEYMOUR BROWNSTEIN,[3,4] ALLAN LISZAUER,[3,4] AND PAUL JOLICOEUR[1,5]*

Laboratory of Molecular Biology[1] and Laboratory of Hematopoiesis and Leukemia,[2] Institut de Recherches Cliniques de Montréal, 110, Avenue des Pins Ouest, Montreal, Quebec H2W 1R7; Departments of Pathology[3] and Ophthalmology,[4] McGill University, Montreal, Quebec H3A 2B4, and Département de Microbiologie et d'Immunologie, Université de Montréal, Montreal, Quebec H3C 3J7,[5] Canada

Received 8 September 1988/Accepted 9 November 1988

Transgenic mice carrying the v-Ha-*ras* oncogene under the control of the mouse mammary tumor virus long terminal repeat were produced. These mice exhibit several phenotypes: mammary tumors, bilateral hyperplasia of the harderian lacrimal gland, primary bronchio-alveolar lung adenocarcinoma, and splenomegaly. High levels of the transgene RNA were detected in mammary, harderian, and lung tumors. Accumulation of cells of the myeloid lineages was found in enlarged spleens. This phenotype may represent an indirect effect of v-Ha-*ras* expression on myeloid progenitors. Our data illustrate the cell-specific effects of v-Ha-*ras*.

The Ha-*ras* proto-oncogene is a member of a larger family of related proteins (Ras family) which have been frequently found activated in several animal and human tumors and are thought to play an important role in the emergence of these malignancies (34). An activated c-Ha-*ras* oncogene has been identified repeatedly in mammary carcinoma induced by *N*-nitroso-*N*-methylurea and by dimethylbenzanthracene in rats (37) and by dimethylbenzanthracene in mice (5). Similarly, activated c-Ha-*ras* or K-*ras* oncogenes have been detected in a number of human breast carcinoma cell lines (17, 18, 23), and some specific Ha-*ras* alleles were found at a higher frequency in patients with breast tumor than in normal patients (19). In fact, most human breast carcinoma revealed elevated levels of c-Ha-*ras* RNA and protein (4, 10, 33). These results suggest a role for this oncogene in the initiation or progression of this malignancy or both.

Transgenic mice offer an attractive system to study the effect of an oncogene in a given tissue under normal physiological conditions. To study the role of the activated v-Ha-*ras* oncogene in the development of mammary tumors, we constructed transgenic mouse lines carrying the v-Ha-*ras* oncogene under the control of the mouse mammary tumor virus (MMTV) enhancer-promoter.

A 4.2-kilobase-pair *Eco*RI-*Nco*I fragment containing the MMTV long terminal repeat (LTR) linked to the v-Ha-*ras* oncogene was purified from pA9 DNA (15) (Fig. 1) and microinjected into the male pronucleus of (C3H × C57BL/6)F_2 zygotes (14). Five animals carrying the transgene (founders) were produced. Four transmitted the transgene to their progeny and were bred as lines (designated lines MR2 to MR5) by mating with BALB/c J mice, as this strain is known to be among the most susceptible to MMTV-induced mammary carcinoma.

Predominant phenotypes of transgenic mice. Five phenotypes not seen in control mice were observed in the transgenic mice: mammary tumors, exophthalmia, splenomegaly, salivary gland tumors, and lung tumors.

Progressive exophthalmia (proptosis) occurred in a significant percentage of animals from three of the five transgenic lines at 2 to 5 months of age (Table 1). It was always bilateral, and the periocular skin became edematous with loss of hair. At dissection, massive enlargement of the retro-orbital harderian gland was observed. Microscopic examination disclosed a papillary and cystic hyperplasia of the alveolar cells of the enlarged normally encapsulated harderian gland (data not shown).

Mammary tumors arose after 4 to 10 months of age in animals of four transgenic lines (Table 1). Each positive mouse generally had multiple tumors of different sizes, arising at different times. Tumors grew slowly but continually, and some reached diameters of over 2 cm. Histological examination of these tumors revealed typical adenocarcinomas at different stages of differentiation and adenoacanthomas (Fig. 2). Three out of four mammary tumors tested could be transplanted in syngeneic or nude mice (data not shown), confirming their malignant nature.

In animals sacrificed because of their multiple mammary and harderian gland tumors or because of their age, a third phenotype, splenomegaly, was observed, mainly in mammary tumor-bearing mice but also in 2 out of 11 (18%) mice which had only harderian gland hyperplasia. Histological examination revealed that the general architecture of the splenic parenchyma was preserved but the red pulp was markedly hypercellular and composed of immature elements of both the granulocytic and erythroid lineages (Table 2). In contrast, differential counts performed on blood and bone marrow cytosmears appeared normal.

To further characterize these cellular populations, spleen and bone marrow cells from transgenic mice sacrificed at late stages of tumor development and from normal mice were plated in methylcellulose cultures to quantify the different hematopoietic precursors (GM-CFC, M-CFC, BFU-E, CFU-GEMM, and CFU-E) (13). A marked increase of all these precursors was detected in the spleens of transgenic mice (Table 3) but not in their bone marrow (data not shown). In these transgenic mice, the splenic stem-cell pool represented a substantial proportion of the total stem cells of the mouse. The splenic hematopoietic progenitors BFU-E,

* Corresponding author.

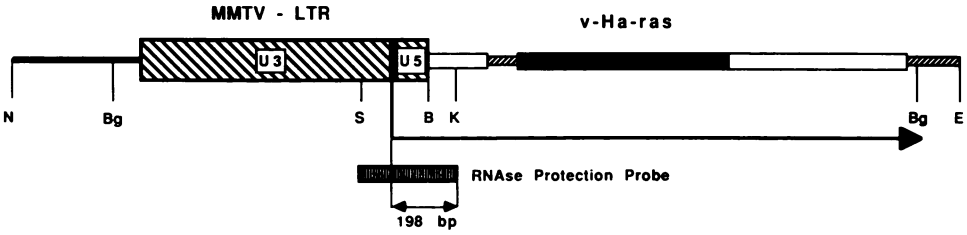

FIG. 1. Schematic representation of the MMTV v-Ha-*ras* fusion gene injected. The v-Ha-*ras* gene under the control of the MMTV LTR was excised from the pA9 plasmid (15) as a 4.2-kilobase-pair *Nco*I-*Eco*RI fragment. A 300-base-pair *Sac*I-*Kpn*I fragment subcloned in Gem-3 was used to generate an antisense RNA probe. —, MMTV *env*; ▨, MMTV LTR; □, 30S sequences; ■, v-Ha-*ras*; ▨, viral sequences; →, expected transcript. The restriction sites are *Bam*HI (B), *Bgl*I (Bg), *Eco*RI (E), *Kpn*I (K), *Nco*I (N), and *Sac*I (S).

GM-CFC, CFU-E, and CFU-GEMM were still dependent on the presence of fetal calf serum for their growth (data not shown). Similarly, all day-7 hematopoietic progenitors (BFU-E, GM-CFC, and CFU-E) were dependent on the presence of IL-3 or erythropoietin for colony formation. In addition, the dose-response curves obtained with erythropoietin for CFU-E colony formation were comparable between cells from normal and transgenic mice (data not shown). The nucleated cells (10^7) from enlarged spleens of four transgenic mice were also tested for their malignant potential by inoculation in nude mice. No tumor growth was detected after a period of observation of 8 weeks.

Mice, predominantly of transgenic line MR5, showed an additional phenotype, lung tumors which appeared as bilateral and multifocal nodules in the lungs at the age of 4 to 13 months. These nodules varied in size (1 to 5 mm in diameter) in each diseased mouse. These lung tumors were found in mice showing other malignancies (mammary adenocarcinoma) but also in mice with no other apparent tumors, suggesting that these tumors were primary tumors of the lung. Histologically, they were diagnosed as typical bronchio-alveolar adenocarcinoma with papillary formation (Fig. 2). Three out of four attempts to transplant these tumors (from four different diseased mice) into nude mice were successful, confirming their malignant nature.

Less frequently, mice from four of the five transgenic lines also developed salivary gland adenocarcinoma (Table 1). The incidence of these tumors was low but significantly higher than that found in normal control mice, strongly suggesting that the v-Ha-*ras* oncogene is also responsible for the initiation or maintenance of these tumors or both.

Tissue-specific expression of the transgene was measured in normal and tumor tissues by an RNase protection assay with an antisense RNA probe (Fig. 1). Elevated levels of the RNA transcripts were detected in all tumors tested: six mammary and four pulmonary tumors and two hyperplastic harderian glands (data not shown). Variable levels of RNA were detected in hyperplastic spleens. High transgene expression was found in some normal tissues such as salivary glands (MR2), harderian glands (MR2), epididymis (MR4B, MR5), seminal vesicle (MR4B and MR5 but not MR2), and testis (MR4B and MR5 but not MR2). Low transgene RNA was also found in other normal tissues such as some spleens (MR2, MR4B, and MR5 but not MR3), thymus (MR2 but not MR4), bone marrow (MR5), lungs (MR2 but not MR3 or MR5), and harderian glands (MR3). Other tissues have not revealed any expression of the transgene: brain (MR2, MR3), heart (MR2), liver (MR2, MR3), kidney (MR2, MR3), ovary (MR2, MR3), uterus (MR2, MR3), and mammary fat pads (MR2, MR5).

Distinct effects of v-Ha-*ras* in various tissues. When expressed in various tissues of transgenic mice, the v-Ha-*ras* oncogene was found to have distinct effects. Alone, v-Ha-*ras* had no transforming ability in any of the tissues where it was expressed. However, in the harderian gland, the expression of v-Ha-*ras* appeared sufficient by itself to stimulate epithelial cell proliferation without inducing malignant transformation (hyperplasia). In these cells, the action of v-Ha-*ras* resembles the mitogenic effect of activated Ha-*ras* or K-*ras* in quiescent cells in vitro; these oncogenes have indeed been found to promote quiescent cells to divide (8, 20). In the mammary glands, the expression of v-Ha-*ras* causes a predisposition to mammary carcinoma but does not seem sufficient by itself for tumor induction, suggesting that additional genetic events (second hit) are required. Together, these results illustrate the cell-specific effect of the v-Ha-*ras* oncogene.

While this work was in progress, Sinn et al. (29) reported similar phenotypes (harderian gland hyperplasia, mammary and salivary gland adenocarcinoma) with the same transgene, although the heterogeneity of types of mammary tumors obtained was not reported. Also, the activated Ha-*ras* oncogene under the control of the enhancer-promoter of the whey acidic protein (*wap*) gene was found to induce

TABLE 1. Pathologies associated with transgenic mice carrying the MMTV v-Ha-*ras* fusion gene

Pathology	% of mice affected (no./total) in line				
	MR2	MR3	MR4A	MR4B	MR5
Harderian gland hyperplasia[a]	92 (72/78)	0 (0/101)	0 (0/22)	87 (13/15)	26 (19/74)
Mammary adenocarcinoma[b]	89 (32/36)	52 (25/48)	0 (0/12)	83 (5/6)	17 (6/36)
Pulmonary adenocarcinoma[c]	6 (1/18)	7 (1/15)	0 (0/6)	0 (0/8)	58 (7/12)
Splenomegaly[c,d]	100 (18/18)	73 (11/15)	0 (0/6)	63 (5/8)	75 (9/12)
Salivary gland adenocarcinoma	5 (4/78)	5 (5/101)	0 (0/22)	7 (1/15)	3 (2/74)

[a] Only data from animals (females and males) older than 2 months were included in this group.
[b] Only data from female mice older than 5 months were included in this group. Only 1 male out of 53 had a mammary tumor in line MR3.
[c] These pathologies were observable at autopsy; thus, only autopsied animals are included in these groups.
[d] Hyperplastic spleen weights ranged from 190 to 1,750 mg and averaged 390 mg. Normal spleen weight was considered to be ≤170 mg.

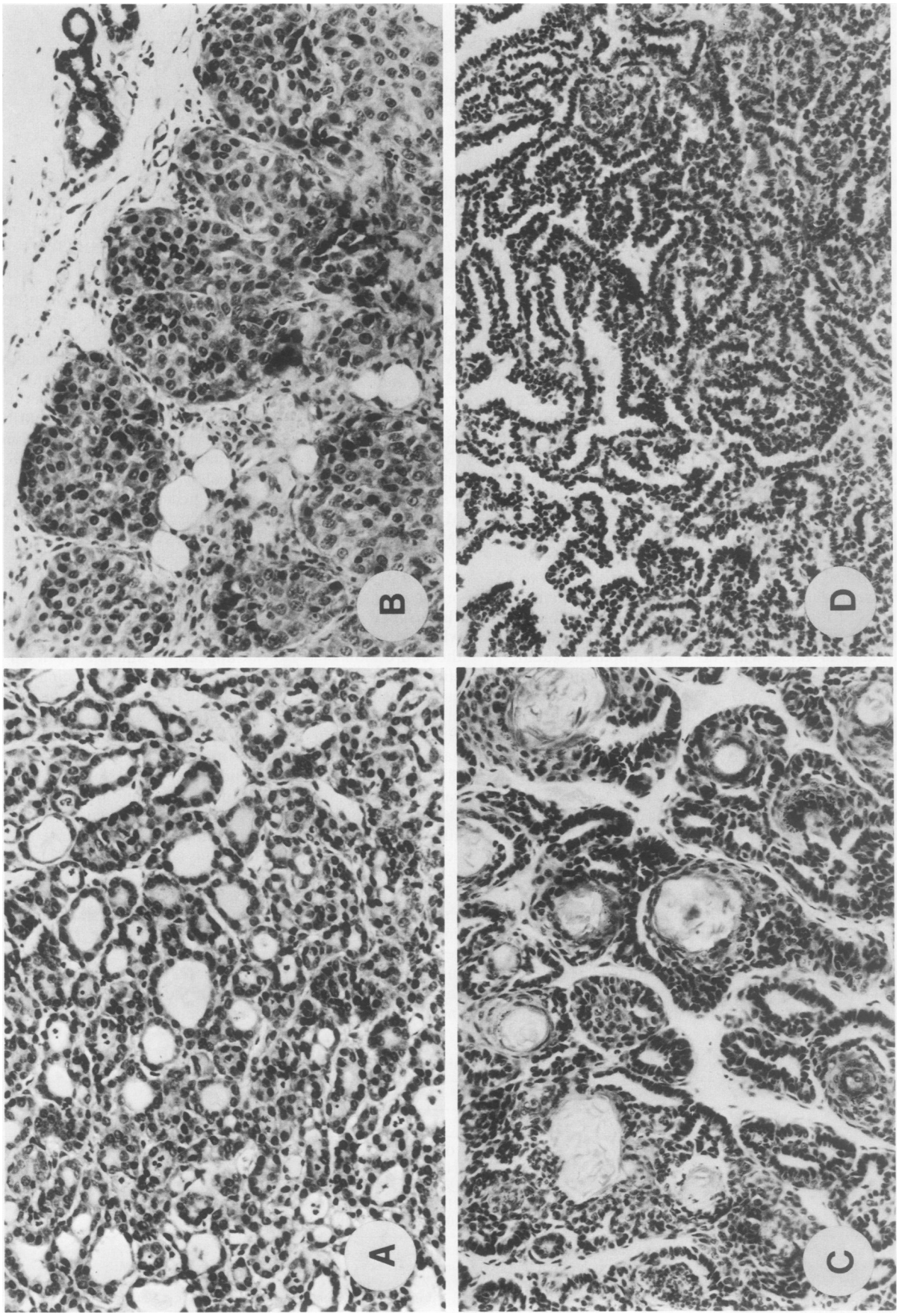

FIG. 2. Mammary and lung adenocarcinomas. (A) Well-differentiated mammary adenocarcinoma with acinar formation. (B) Less-differentiated mammary tumor with no glandular structure; nonneoplastic breast tissue is present (upper right). (C) Mammary adenoacanthoma showing both glandular formation and squamous differentiation with keratin pearl formation. (D) Lung tumor with neoplastic cells arranged in columns and papillary formations. Hematoxylin and eosin stain; magnification, ×175.

TABLE 2. Cellular compositions of spleens and bone marrow from transgenic and normal mice

Cell type[a]	Percentage of cells from:							
	Spleen					Bone marrow		
	Transgenic			Normal		Transgenic		Normal
	T7	T8	T9	N4	N5	T7	T9	N4
Blasts	5	3	3	0	0	3	2	1
Promyelocytes; myelocytes	15	13	10	0	0	7	2	5
Metamyelocytes; polymorphonuclear leukocytes	20	54	45	3	3[b]	69	71	68
Lymphocytes	41	24	30	93	95	9	16	21
Erythroblasts	9	3	3	0	0	3	2	2
Monocytes	6	3	4	2	1	5	1	2
Eosinophils	3	0	4	2	1[b]	3	7	5

[a] At least 300 cells were counted for each morphological determination.
[b] A parasitic infection in the colony at the time of the assay could account for the low percentage of mature neutrophils and eosinophils found in the spleens of normal mice.

salivary gland and mammary adenocarcinoma in transgenic mice, but at a much lower frequency (1).

The splenomegaly observed in our transgenic mice was unexpected and represents a complex phenotype, surprisingly restricted to the spleen and not found in the bone marrow. This disease resembles human myelodysplasia or myeloid metaplasia. Interestingly, activation of genes of the Ras family has been reported in human chronic myeloproliferative disorders and myelodysplastic syndromes (12, 16). This splenomegaly phenotype was not reported by Sinn et al. (29) in the mice carrying the same transgene and presenting mammary and harderian gland phenotypes. Some observations that favor the interpretation that this phenotype is unrelated to v-Ha-*ras* expression are as follows. (i) Although the expression of genes under the control of MMTV LTR in the spleen is well documented (3, 29, 31), especially in BALB/c mice (27), the levels of v-Ha-*ras* transcripts in our transgenic mice were found to be often relatively low in the spleen and did not seem to correlate with the degree of splenomegaly. However, high levels of expression of the transgene restricted to a small subpopulation of spleen cells would not have been detected by the technique used. (ii) Splenomegaly was also observed in C3H/OuJ mice carrying MMTV-induced mammary tumors, suggesting that it might represent a paraneoplastic phenotype. However, other facts favor the interpretation that this phenotype is related to the expression of the transgene. (i) Among the transgenic mice, splenomegaly was observed only in lines expressing the transgene and some tumor phenotype. (ii) Splenomegaly was not observed in normal BALB/c littermates caged in the same room. (iii) Splenomegaly was also observed in a few macroscopic malignant tumor-free transgenic mice from lines expressing the transgene (MR2, MR4B). The genetic background may be responsible for the appearance of this additional phenotype reported here. If splenomegaly is the result of the transgene effect, our results are best explained by postulating that the expression of v-Ha-*ras* in a specific spleen cell subpopulation (possibly stromal cells) leads to the secretion of a factor stimulating all myeloid progenitors.

Lung tumors in transgenic mice. Lung tumors arose at a significant frequency in transgenic mice of line MR5. This finding was unexpected, since lung tumors were not reported by Sinn et al. (29), despite expression of the transgene in the lungs of their transgenic mice. Again, as with the splenomegaly, the genetic background of the mice may be critical for the outcome of the disease. In mice, susceptibility to lung tumors is primarily controlled by genetic influence, and the BALB/c mice used in our experiments are relatively susceptible (25).

There are good reasons to believe that these tumors are primary tumors of the lung and not metastases. (i) They have been found in two tumor-free male animals. (ii) The pathology is unique and distinct from that of the mammary adenocarcinoma analyzed. (iii) These tumors are pathologically indistinguishable from the spontaneous lung tumors of the A/J mice classified as adenocarcinoma (9).

Lung tumors, which arose relatively late and stochastically, are most likely dependent on v-Ha-*ras* expression for initiation or maintenance or both, since very high levels of the transgene RNA were detected in all tumors tested (4 of 4), while low or undetectable levels of transcripts were found in the normal transgenic lungs. However, measurement of RNA transcripts from cells of the total organ would not be sensitive enough to detect them appropriately in a rare-cell population such as the epithelial cells lining the bronchioles, a cell population previously found to express transcripts under the control of MMTV LTR (30). However, although the expression of v-Ha-*ras* in some cells in the lung predisposes them to transformation, it does not appear sufficient,

TABLE 3. Hematopoietic progenitors in spleens of transgenic and normal mice

Progenitor	Total no. of progenitors in spleen[a]							
	Transgenic					Normal		
	T1	T2	T3	T6	T7	N1	N2	N3
CFU-E	135,600	1,000,000	200,000	1,950,000	640,000	36,100	nd[b]	21,000
BFU-E	7,600	23,500	5,000	32,800	8,300	1,500	200	1,000
CFU-GEMM	1,600	7,000	2,700	5,800	5,200	500	200	200
GM-CFC	9,400	29,200	23,000	125,600	56,000	3,600	800	1,700
M-CFC	13,600	nd[b]	9,700	32,100	11,200	2,400	600	1,200
Sex of mouse	M	F	M	F	F	M	F	M
Age (mo)	5.5	8.0	5.0	8.5	13.0	4.0	10.0	10.0

[a] Total number of progenitors per spleen was obtained by multiplying the frequency of each type of progenitor by the total number of nucleated cells recorded after dispersion of spleen cells by homogenization.
[b] nd, Not determined.

and an additional genetic event(s) (second hit), as well as the presence of appropriate susceptibility genes, is most likely required to fully transform these lung cells. Interestingly, a good correlation has been shown between a K-*ras*-2 *Eco*RI polymorphism and the differential susceptibility of mice to development of pulmonary adenocarcinoma (25). N-*ras* has also been found to be activated in a Lewis lung carcinoma (35). Recently, lung tumors have also been reported to arise in transgenic mice carrying the v-Ha-*ras* oncogene under the simian virus 40 promoter linked to the human immunoglobulin heavy-chain enhancer (32). Furthermore, activation of *ras* proto-oncogenes has been reported in human lung tumors (7, 24) or lung tumor cell lines (2, 6, 21, 22, 26, 28, 36), and rare alleles of Ha-*ras*-1 gene have been found in patients with non-small-cell carcinomas as compared with normal patients or with patients with small-cell carcinoma (11). Therefore, our lung tumors represent an attractive and novel model to study spontaneously arising lung tumors in animals.

This work was supported by grants from the Medical Research Council of Canada (MRC) and from the National Cancer Institute of Canada (NCI) to P.J. Work performed in the laboratory of T.H. was also supported by grants from the MRC and NCI of Canada. P.J.T. was the recipient of a studentship from the MRC of Canada.

We thank Christine Mayer and André Haman for their excellent technical assistance and Marie Bernier for preparing the manuscript. We are most grateful to Robert Sawka for kindly performing immunohistochemistry studies on tissue sections. We thank Gordon Hager for supplying the pA9 clone and R.A. Philipps for performing B-cell progenitor assays. We thank Alan Peterson (Ludwig Institute, Montreal) for initiating us to the transgenic mouse system and Helmut Zarbl for his interest in initiating this project.

LITERATURE CITED

1. **Andres, A. C., C. A. Schönenberger, B. Grower, L. Hennighausen, M. Lemeur, and P. Gerlinger.** 1987. Ha-ras oncogene expression directly by a milk protein gene promoter: tissue specificity, hormonal regulation, and tumor induction in transgenic mice. Proc. Natl. Acad. Sci. USA **84**:1299–1303.
2. **Capon, D. J., P. H. Seeburg, J. P. McGrath, J. S. Hayflick, U. Edman, A. D. Levinson, and D. V. Goeddel.** 1983. Activation of Ki-ras 2 gene in human colon and lung carcinomas by two different point mutations. Nature (London) **304**:507–513.
3. **Choi, Y., D. Henrard, I. Lee, and S. R. Ross.** 1987. The mouse mammary tumor virus long terminal repeat directs expression in epithelial and lymphoid cells of different tissues in transgenic mice. J. Virol. **61**:3013–3019.
4. **Clair, T., W. R. Miller, and Y. S. Cho-Chung.** 1987. Prognostic significance of the expression of a ras protein with a molecular weight of 21,000 by human breast cancer. Cancer Res. **47**: 5290–5293.
5. **Dandekar, S., S. Sukumar, H. Zarbl, L. J. T. Young, and R. D. Cardiff.** 1986. Specific activation of the cellular Harvey-ras oncogene in dimethylbenzanthracen-induced mouse mammary tumors. Mol. Cell. Biol. **6**:4104–4108.
6. **Der, C. J., and G. M. Cooper.** 1983. Altered gene products are associated with activation of cellular ras K genes in human lung and colon carcinomas. Cell **32**:201–208.
7. **Eva, A., S. R. Tronick, A. Gol, J. H. Pierce, and S. Aaronson.** 1983. Transforming genes of human hematopoietic tumors: frequent detection of ras-related oncogenes whose activation appears to be independent of tumor phenotypes. Proc. Natl. Acad. Sci. USA **80**:4926–4930.
8. **Feramisco, J., M. Gross, T. Kamato, M. Rosenberg, and R. Sweet.** 1984. Microinjection of the oncogenic form of the human H-ras (T-24) protein results in rapid proliferation of quiescent cells. Cell **38**:109–117.
9. **Green, E. L.** 1968. Biology of the laboratory mouse, p. 534. 2nd ed. Dover Publications, Inc., N.Y.
10. **Hand, P. H., V. Vilasi, A. Thor, N. Ohuchi, and J. Schlom.** 1987. Quantitation of Harvey ras p21-enhanced expression in human breast and colon carcinoma. J. Natl. Cancer Inst. **79**:59–65.
11. **Heighway, J., N. Thatcher, T. Cerny, and P. S. Hasleton.** 1986. Genetic predisposition to human lung cancer. Br. J. Cancer **53**:453–457.
12. **Hirai, H., Y. Kobayashi, H. Mano, K. Hagiwara, Y. Maru, M. Omine, H. Mizoguchi, J. Nishida, and F. Takaku.** 1987. A point mutation at codon 13 of the N-ras oncogene in myelodysplastic syndrome. Nature (London) **327**:430–432.
13. **Hoang, T., D. Gilmore, D. Metcalf, S. Cobbold, S. Watt, M. Clark, M. Furth, and H. Waldmann.** 1983. Separation of hemopoietic cells from adult bone marrow by use of monoclonal antibodies. Blood **61**:580–588.
14. **Hogan, B., F. Costantini, and E. Lacy.** 1986. Manipulating the mouse embryo: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Huang, A. L., M. C. Ostrowski, D. Bérard, and G. L. Hager.** 1981. Glucocorticoid regulation of the Ha-MuSV p21 gene conferred by sequences from mouse mammary tumor virus. Cell **27**:245–255.
16. **Janssen, J. W. G., A. C. M. Steenvoorden, J. Lyons, B. Anger, J. U. Böhlke, J. L. Bos, H. Seliger, and C. R. Bartram.** 1987. Ras gene mutations in acute and chronic myelocytic leukemias, chronic myeloproliferative disorders, and myelodysplastic syndromes. Proc. Natl. Acad. Sci. USA **84**:9228–9232.
17. **Kozma, S. C., M. E. Bogaard, K. Buser, S. M. Saurer, J. L. Bos, B. Grower, and N. E. Hynes.** 1987. The human c-Kirsten ras gene is activated by a novel mutation in codon 13 in the breast carcinoma cell line MDA-MB 231. Nucleic Acids Res. **15**: 5963–5971.
18. **Kraus, M. H., Y. Yuasa, and S. A. Aaronson.** 1984. A position 12-activated H-ras oncogene in all HS578T mammary carcinoma cells but not normal mammary cells of the same patient. Proc. Natl. Acad. Sci. USA **81**:5384–5388.
19. **Lidereau, R., C. Escot, C. Theillet, M. H. Champeme, M. Brunet, J. Gest, and R. Callahan.** 1986. High frequency of rare alleles of the human c-Ha-ras-1 protooncogene in breast cancer patients. J. Natl. Cancer Inst. **77**:697–701.
20. **Lumpkin, C. K., J. E. Knepper, J. S. Butel, J. R. Smith, and O. M. Pereira-Smith.** 1986. Mitogenic effects of the proto-oncogene and oncogene forms of c-Ha-ras DNA in human diploid fibroblasts. Mol. Cell. Biol. **6**:2990–2993.
21. **McCoy, M. S., J. J. Toole, J. M. Cunningham, E. H. Chang, D. R. Lowy, and R. A. Weinberg.** 1983. Characterization of a human colon/lung carcinoma oncogene. Nature (London) **302**: 79–81.
22. **Nakano, H., F. Yamamoto, C. Neville, D. Evans, T. Mizuno, and M. Perucho.** 1984. Isolation of transforming sequences of two human lung carcinomas: structural and functional analysis of the activated c-K-ras oncogenes. Proc. Natl. Acad. Sci. USA **81**:71–75.
23. **Prosperi, M. T., J. Even, F. Calvo, J. Lebeau, and G. Goubin.** 1987. Two adjacent mutations at position 12 activate the K-ras 2 oncogene of a human mammary tumor cell line. Oncogene Res. **1**:121–128.
24. **Rodenhuis, S., M. L. van de Wetering, W. J. Mooi, S. G. Evers, N. van Zandwijk, and J. L. Bos.** 1987. Mutational activation of the K-ras oncogene: a possible pathogenetic factor in adenocarcinoma of the lung. N. Engl. J. Med. **317**:929–935.
25. **Ryan, J., P. E. Barker, M. N. Nesbitt, and F. H. Ruddle.** 1987. K-ras-2 as genetic marker for lung tumor susceptibility in inbred mice. J. Natl. Cancer Inst. **79**:1351–1357.
26. **Santos, E., D. Martin-Zanca, E. Premkumar Reddy, M. A. Pierotti, G. Della Porta, and M. Barbacid.** 1984. Malignant activation of c-K-ras oncogene in lung carcinoma but not in normal tissue of the same patient. Science **223**:661–664.
27. **Sarkar, A., and W. H. Gunzburg.** 1986. Spleen specific expression of an MMTV related transcript associated with the MTV-6 locus in BALB/c mice. Virology **154**:233–239.
28. **Shimizu, K., D. Birbaum, M. A. Ruley, O. Fasano, Y. Suard, L. Edlund, E. Taparowsky, M. Goldfarb, and M. Wigler.** 1983.

Structure of the Ki-ras gene of the human lung carcinoma cell line Calu-1. Nature (London) **304**:497–500.

29. **Sinn, E., W. Muller, P. Pattengale, I. Tepler, R. Wallace, and P. Leder.** 1987. Coexpression of MMTV/v-Ha-ras and MMTV/c-myc genes in transgenic mice: synergistic action of oncogenes in vivo. Cell **49**:465–475.
30. **Stewart, T. A., P. G. Hollingshead, and S. L. Pitts.** 1988. Multiple regulatory domains in the mouse mammary tumor virus long terminal repeat revealed by analysis of fusion genes in transgenic mice. Mol. Cell. Biol. **8**:473–479.
31. **Stewart, T. A., P. K. Pattengale, and P. Leder.** 1984. Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/*myc* fusion genes. Cell **38**:627–637.
32. **Suda, Y., S. Aizawa, S. I. Hirai, T. Inoue, Y. Furuta, M. Suzuki, S. Hirohashi, and Y. Ikawa.** 1987. Driven by the same Ig enhancer and SV40 T promoter ras induced lung adenomatous tumors, myc induced pre-B cell lymphomas and SV40 large T gene a variety of tumors in transgenic mice. EMBO J. **16**: 4055–4065.
33. **Theillet, C., R. Lidereau, C. Escot, P. Hutzell, M. Brunet, J. Gest, J. Schlom, and R. Callahan.** 1986. Loss of c-Ha-ras 1 allele and aggressive human primary breast carcinomas. Cancer Res. **46**:4776–4781.
34. **Varmus, H. E.** 1984. The molecular genetics of cellular oncogenes. Annu. Rev. Genet. **18**:553–612.
35. **Vousden, K. H., and C. J. Marshall.** 1984. Three different activated ras genes in mouse tumors; evidence for oncogene activation during progression of a mouse lymphoma. EMBO J. **3**:913–917.
36. **Yuasa, Y., R. A. Gol, A. Chang, I. M. Chiu, E. Premkumar Reddy, S. R. Tronick, and S. A. Aaronson.** 1984. Mechanism of activation of an N-ras oncogene of SW-1271 human lung carcinoma cells. Proc. Natl. Acad. Sci. USA **81**:3670–3674.
37. **Zarbl, H., S. Saraswati, A. V. Arthur, D. Martin-Zanca, and M. Barbacid.** 1985. Direct mutagenesis of Ha-ras-1 oncogenes by N-nitroso-N-methylurea during initiation of mammary carcinogenesis in rats. Nature (London) **315**:382–385.