Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 3070–3074, May 1989
Biochemistry

Activation of the Ki-*ras* protooncogene in spontaneously occurring and chemically induced lung tumors of the strain A mouse

(lung adenoma/lung adenocarcinoma/point mutations/chemical carcinogens)

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Communicated by James A. Miller, January 17, 1989 (received for review October 26, 1988)

ABSTRACT **The strain A mouse has a high incidence of spontaneous lung tumors and is susceptible to lung tumor induction by chemical carcinogens. By utilizing transfection assay, Southern blot analysis, and DNA amplification techniques, we have detected an activated Ki-*ras* gene in the DNAs of both spontaneously occurring and chemically induced lung tumors of strain A mice. The point mutations in the spontaneous lung tumors were in both codon 12 (60%) and codon 61 (30%). In contrast, 100% of the mutations in the Ki-*ras* gene detected in methylnitrosourea-induced lung tumors and 93% of the mutations in the Ki-*ras* genes detected in benzo[*a*]pyrene-induced lung tumors were in codon 12, whereas 90% of the mutations in the Ki-*ras* genes detected in ethyl carbamate-induced lung tumors were in codon 61. The selectivity of mutations in the Ki-*ras* oncogene observed in chemically induced tumors, as compared to spontaneous tumors, suggests that these chemicals directly induce point mutations in the Ki-*ras* protooncogene. These data indicate that the strain A mouse lung tumor model is a very sensitive system to detect the ability of chemicals to activate the Ki-*ras* protooncogene in lung tissue.**

The strain A mouse lung tumor bioassay has been utilized for testing more than 300 compounds for carcinogenic activity (1–4). The strain A mouse also has a very high incidence of spontaneous lung tumors (nearly 100% by 2 years of age), which is often a complicating factor in interpreting bioassay test results for carcinogenicity (1–4). The detection and characterization of activated oncogenes involved in the development of both spontaneously occurring and chemically induced lung tumors of the strain A mouse may offer one approach to define the mechanism(s) by which a chemical causes an increased incidence of lung tumors. Activated *ras* oncogenes have been detected in spontaneously occurring and chemically induced liver tumors of the B6C3F1 mouse (5–8) and the pattern of activating mutations in these tumors has been compared. More than 60% of the activated oncogenes detected in furan- and furfural-associated liver tumors of the B6C3F1 mouse were different from those seen in spontaneous B6C3F1 mouse liver tumors. Activating mutations in the Ha-*ras* gene and several activated Ki-*ras* genes were detected in chemically induced mouse liver tumors, indicating that furan and furfural caused an increase in mouse liver tumors at least in part by a genotoxic mechanism (7). Likewise, Wiseman *et al.* (8) demonstrated that the activated Ha-*ras* gene observed in chemically induced mouse liver tumors resulted directly from reaction of the ultimate carcinogens with this gene. The present study systematically analyzed oncogene activation in both spontaneously occurring and chemically induced lung tumors in a classical and widely used rodent model. Possible mechanism(s) of tumor induction by benzo[*a*]pyrene (B[*a*]P), ethyl carbamate (EC), and methylnitrosourea (MNU) were determined through comparison of protooncogene activation between spontaneous and chemically induced lung tumors. The results provide important information to assess the usefulness of the strain A mouse lung as a rodent model to investigate the corresponding human neoplasm.

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MATERIALS AND METHODS

Rodent Tumors. Spontaneous lung tumors were obtained from untreated, 24- to 27-month-old strain A/HeN mice (National Institute of Aging, Bethesda, MD). Strain A/J mice (6–8 weeks old, The Jackson Laboratories) were administered B[*a*]P by oral intubation at 3 mg per dose, once a week, for 4 weeks (9). EC and MNU were administered i.p. in a single injection with a dose of either 1000 mg/kg of body weight (for EC) or 150 mg/kg of body weight (for MNU) (10). At 12 months, individual lung tumors were collected and quick frozen in liquid nitrogen. A representative portion of both the spontaneous and chemically induced tumors, when available, was fixed in 10% (vol/vol) neutral buffered formalin for histopathological examination.

Transfection Assays. High molecular weight DNA was isolated from normal and tumor tissues and DNA transfection analysis was carried out by the protocol described (6).

Southern Blot Analysis. This was performed as described (6). High molecular weight DNA was isolated, digested with restriction enzymes, and separated by electrophoresis in a 0.7% agarose gel. The DNA was then transferred to a nitrocellulose sheet. The probe was a 0.618-kilobase *Sst* II–*Hin*cII fragment of the v-Ki-*ras* gene (Oncor, Gaithersburg, MD).

DNA Amplification. Amplification reactions were carried out essentially as described (11). Briefly, a 100-μl reaction mixture containing 1 μg of genomic DNA in 50 mM KCl/10 mM Tris, pH 8.4/2.5 mM $MgCl_2$, with each primer at 1 μM, 200 μM dATP, 200 μM dCTP, 200 μM TTP, and 200 μM dGTP, gelatin at 200 μg/ml, and 2 units of *Thermus aquaticus* (*Taq*) polymerase (Perkin–Elmer/Cetus) was overlaid with several drops of paraffin oil to prevent evaporation and subjected to 25 cycles of amplification as follows: the samples were heated to 95°C for 1 min, cooled to 37°C for 2 min, and incubated at 72°C for 3 min.

Direct Sequencing of Amplified DNAs. Procedures for direct sequencing of the amplified Ki-*ras* exons were as described (12).

Slot-Blot Oligonucleotide Hybridizations. Amplified DNA samples (5 ng) were mixed with 100 μl of 0.4 M NaOH/25 mM EDTA and applied to Nytran nylon filters (Schleicher & Schuell) using a slot-blot apparatus (Schleicher & Schuell). The filters were hybridized and washed according to the method of Verlaan-de Vries *et al.* (13).

Abbreviations: B[*a*]P, benzo[*a*]pyrene; EC, ethyl carbamate; MNU, methylnitrosourea; PCR, polymerase chain reaction.

RESULTS

Histology of Strain A Mouse Lung Tumors. The published criteria for diagnosis of lung tumors were followed (14). Lung tumors in both spontaneously occurring and chemically treated mice were morphologically similar to those described (2–4). Five of the spontaneous tumors were diagnosed as lung adenocarcinomas. The other 6 spontaneous tumors were designated as probable lung adenomas. All 16 B[*a*]P-induced lung tumors were available for histology and were all diagnosed as adenomas. Due to the small tumor size, only 7 of 11 EC-induced lung tumors were available for histology; 5 were diagnosed as adenocarcinomas; 2 were diagnosed as adenomas. The four EC-induced lung tumors without histology were diagnosed as lung adenoma based on their small size (15). All 15 of the MNU-induced tumors were available for histology and were diagnosed as adenomas.

Detection of Transforming Genes in Chemically Induced Lung Tumors. Spontaneous lung tumors and B[*a*]P- and EC-induced lung tumors were examined for the presence of activated oncogenes. As shown in Table 1, transforming genes were detected in the DNAs from both spontaneously occurring tumors and chemically induced tumors. DNA from 10 of 11 spontaneous tumors, 14 of 16 B[*a*]P-induced tumors, and 9 of 10 EC-induced tumors scored as positive for focus formation in the NIH 3T3 transfection assay. The transforming efficiencies of the DNAs from the chemically induced tumors were comparable to the transforming efficiencies seen with spontaneous tumor DNAs of the strain A mouse (Table 1). No foci were observed when NIH 3T3 cells were transfected with DNA isolated from 24-month-old and 12-month-old morphologically normal lung tissue from untreated and chemically treated strain A mice, respectively (Table 1).

Identification of Oncogenes in the Lung Tumors. Primary transfectant DNAs were analyzed by Southern blotting and hybridization with probes specific for Ki-*ras* by DNA transfection techniques. DNAs from foci induced by 10 spontaneous lung tumors (Fig. 1*A*, lanes 2–11), 14 B[*a*]P-induced lung tumor DNAs (data not shown), and 10 EC-induced lung tumor DNAs (data not shown) exhibited an increase in hybridization intensity and/or rearranged Ki-*ras* DNA fragments. These findings strongly suggest that these transformants had incorporated tumor-derived alleles of the Ki-*ras* gene.

Table 1. Transforming genes in spontaneously occurring and chemically induced lung tumors of the strain A mouse

DNA source	Samples tested, no.	Frequency, no. positive/ no. tested		Transformation efficiency, foci per μg of DNA
Untreated normal lung*	10	0/10	(0)	—
Chemically treated normal lung†	30	0/30	(0)	—
Spontaneous				
Lung adenomas	6	5/6	(83)	0.025–0.058
Lung adenocarcinomas	5	5/5	(100)	0.008–0.050
B[*a*]P-induced lung adenomas	16	14/16	(87)	0.004–0.058
EC-induced				
Lung adenomas	6	5/6	(83)	0.017–0.058
Lung adenocarcinomas	5	5/5	(100)	0.025–0.058

Numbers in parentheses are percentage of positive tumors.
*Five lung tissue samples from 2-month-old and five lung tissue samples from 24-month-old untreated mice in which no tumors were observed.
†Ten lung tissue samples from 12-month-old mice, each group treated with B[*a*]P or with EC in which no tumors were observed, or 10 lung tissue samples from 6- to 8-week-old mice.

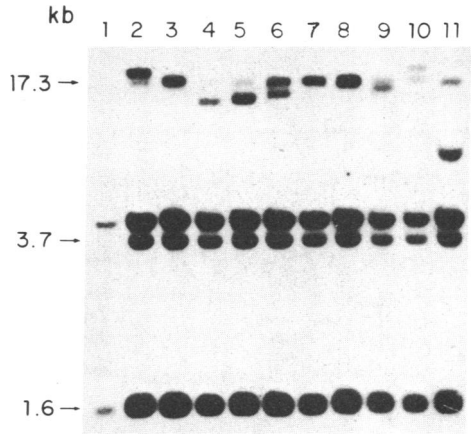

FIG. 1. Southern blot analysis of Ki-*ras* sequences in NIH 3T3 transformants derived from spontaneous lung tumors. *Hin*dIII-digested DNA (20 μg) was electrophoresed on an agarose gel, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled probe. Lanes: 1, NIH 3T3 DNA; 2–11, various transformant DNAs. kb, Kilobases.

Sequence Analysis of the Activating Mutations in Ki-*ras* Gene. Activated *ras* genes with point mutations in their coding sequences frequently produce p21 proteins with altered eletrophoretic mobilities. Slower or faster electrophoretic mobilities appear to accompany many of the activating lesions at codons 12 and 61, respectively (6–8). The p21 proteins in the NIH 3T3 cells transformed with DNA derived from spontaneous tumors or from B[*a*]P- or EC-induced tumors exhibited either faster or slower than normal electrophoretic mobilities (data not shown). The samples were first analyzed for mutations in codons 12 or 61. The point mutations were identified by utilizing polymerase chain reaction (PCR) technique to amplify the exons of the Ki-*ras* gene followed by sequence analysis of amplified product. Three sets (each set including two oligomers) of PCR primers and three sequencing primers for exons 1, 2, and 3 were synthesized according to the published mouse Ki-*ras* gene sequences (16). Exon 1 (spanning a region including codons 12 and 13), exon 2 (spanning the codon 61 region), and exon 3 (spanning the region of codons 116–119) of the Ki-*ras* gene were amplified for each individual transfectant and control DNA. The sequences were determined and the results (representative mutations) are shown in Fig. 2 *a–g*.

Sequence analysis indicated that single-base mutations had occurred at either codon 12 or codon 61 in both the spontaneously occurring and the B[*a*]P- and EC-induced lung tumor DNAs. Six of nine mutations in the Ki-*ras* gene of spontaneous lung tumors were in codon 12 with three of six mutations having a G·C → A·T transition, two of six mutations having a G·C → T·A transversion, both at the second position, and one of six mutations having a G·C → C·G transversion at the first position of codon 12. Three of nine mutations in the Ki-*ras* gene of spontaneous tumors were in codon 61 with two of three mutations having an A·T → G·C transition at the second position and one of three mutations having an A·T → T·A transversion at the third position of codon 61.

Mutations in the Ki-*ras* gene of B[*a*]P-induced lung tumors were in codon 12 with 9 of 13 mutations having a G·C → T·A transversion at the first (8/9) or the second (1/9) positions of codon 12 and 4 of 13 mutations having a G·C → A·T transition at the second position of codon 12. Ninety percent of the mutations in the Ki-*ras* gene of EC-induced lung tumors were in codon 61 (9/10) with 7 of 9 mutations having an A·T → T·A

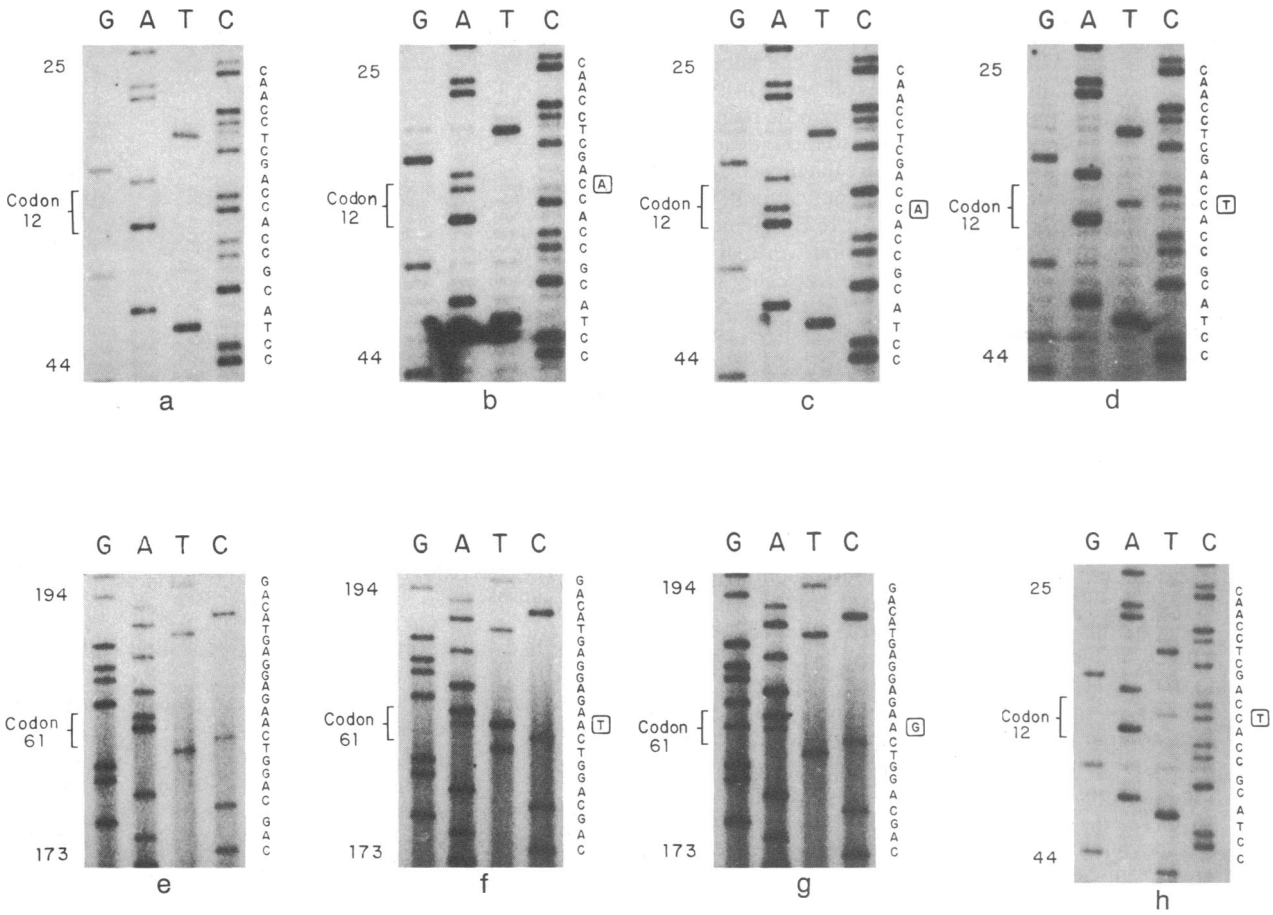

FIG. 2. Identification of the codon 12 and the codon 61 mutations by direct sequencing of PCR-amplified DNA. Oligonucleotides were synthesized for PCR and exons 1 and 2 of the Ki-*ras* gene were directly sequenced. The PCR primers were 5′-ATGACTGAGTATAAACTTGT-3′/5′-CTCTATCGTAGGGTCGTACT-3′ for exon 1, 5′-GACTCCTACAGGAAACAAGT-3′/5′-CTATAATGGTGAATATCTTC-3′ for exon 2, and 5′-AGAACAAATTAAAAGAGTAA-3′/5′-CTGTCTTGTCTTTGCTGAGG-3′ for exon 3. The sequencing primers were 5′-AAGTGATTCTGAATTAGCTG-3′ (sequencing the antisense strand) for exon 1 covering codon 12, 5′-AATTGATGGAGAAACCTGTC-3′ (sequencing the sense strand) for exon 2 covering codon 61, and 5′-AAAGGACTCTGAAGATGTGC-3′ (sequencing the sense strand) for exon 3 covering codons 116–119. The representative autoradiograms of sequence analyses are shown. Sequences are shown on the left and mutations are noted by the nucleotides enclosed in boxes on the right. Lanes (from left to right) are for the bases guanine (G), adenine (A), thymine (T), and cytosine (C). (*a*) Exon 1 of normal lung tissue DNA. (*b–d*) Exon 1 of transformant DNAs. (*e*) Exon 2 of normal lung tissue DNA. (*f* and *g*) Exon 2 of transformant DNAs. (*h*) Exon 1 of MNU-induced tumor DNA.

transversion at the second position of codon 61 and 2 of 9 mutations having an A·T → G·C transition at the second position of codon 61. The remaining EC-induced tumor had an A·T → G·C transition at the second position of codon 12.

All the p21 proteins of activated Ki-*ras* gene with a mutation in codon 61 migrated faster than the normal proteins, whereas the p21 proteins of activated Ki-*ras* gene with a mutation in codon 12 migrated slower relative to mouse endogenous p21 bands on SDS/polyacrylamide gels (data not shown). The correlation between the altered electrophoretic mobilities of the p21 proteins of the Ki-*ras* gene and the site of the mutations in the Ki-*ras* gene was similar to that observed (6–8) for p21 proteins of activated Ha-*ras* gene detected in mouse liver tumors. The mutation in the Ki-*ras* oncogene from one of the spontaneous tumors and from one of the B[*a*]P-induced tumors has not been identified, but was not in codons 12, 13, 61, or 116–119. However, the p21 *ras* proteins from these two transformants migrated much slower than the p21 *ras* proteins of activated Ki-*ras* gene with a mutation in codon 12. Reynolds *et al.* (7) also detected two activated Ki-*ras* genes in mouse liver tumors whose p21 proteins migrated in a very similar manner (figure 4A, lanes f and j, of ref. 7).

The patterns of mutations in the Ki-*ras* oncogene detected in spontaneous tumors and B[*a*]P- and EC-induced tumors are summarized in Table 2. The activating mutations identified by PCR-direct sequencing were confirmed by selective oligonucleotide hybridization using transformant DNAs (data not shown). A new *Xba* I restriction site was detected in the Ki-*ras* gene of seven EC-induced lung tumor DNA transformants (data not shown). The new *Xba* I site resulted from the A·T → T·A transversion at the second position of codon 61 (Table 2). Several original tumor DNA samples were also analyzed by PCR-direct sequencing technique. In each case, the mutation in the tumor DNA was the same as mutation in the transformant DNA.

Analysis of Mutations in the Ki-*ras* Gene of MNU-Induced Lung Tumors. MNU activated the Ha-*ras* gene by a G·C → A·T transition at the second base of codon 12 in rat mammary tumors (17, 18). Since activated Ki-*ras* genes were detected in all of the NIH 3T3 transformants derived from DNA from spontaneous tumors and B[*a*]P- and EC-induced tumor, MNU-induced lung tumor DNAs were directly analyzed for the presence of the G·C → A·T transition at the second position of codon 12 in the Ki-*ras* gene of strain A mice. Normal lung and tumor DNA samples amplified by the PCR procedure were screened for hybridization to two 19-mers: one was homologous to the wild-type sequence at codon 12 (wild-type codon 12 probe); the other had a G → A base change at the second position of codon 12 (mutant codon 12

Table 2. Mutation spectrum in Ki-*ras* genes in lung tumors of the strain A mouse

Tumor	Activated Ki-*ras*, no.	Mutations, no.							
		Codon 12				Codon 61			
		TGT	GTT	GAT	CGT	CTA	CGA	CAT	Unknown
Spontaneous									
Adenomas	5	0	2	1	1	0	0	0	1
Adenocarcinomas	5	0	0	2	0	0	2	1	
B[*a*]P-induced adenomas	14	8	1	4	0	0	0	0	1
EC-induced									
Adenomas	5	0	0	0	0	4	1	0	0
Adenocarcinomas	5	0	1	0	0	3	1	0	0
MNU-induced adenomas	15	0	0	15	0	0	0	0	0

Normal codon 12 is GGT encoding glycine; normal codon 61 is CAA encoding glutamine. TGT encodes cysteine; GTT encodes valine; GAT encodes aspartic acid; CGT encodes arginine; CTA encodes leucine; CGA encodes arginine; CAT encodes histidine.

probe). The results of the hybridization experiments are shown in Fig. 3. The DNA samples from both the normal lung tissue and MNU-induced lung tumors hybridized to the wild-type codon 12 probe. However, only DNA samples from MNU-induced lung tumors (15/15 tumors) hybridized to the mutant codon 12 probe under stringent conditions, indicating a G·C → A·T transversion at the second position of codon 12 in each tumor. The same results were observed by direct sequence analysis of PCR-amplified DNAs from MNU-induced lung tumors (one example is shown in Fig. 2*h*). Both the normal allele and mutated allele of the Ki-*ras* gene were observed at the second position of codon 12. The intensity of the cytosine band (normal allele) was slightly stronger than the thymine band (mutated allele), which probably reflects the presence of some normal cells in the tumor tissue.

Some of the MNU-induced tumors were very small (<1 mm in diameter). Analysis of tumors by transfection assay was not possible. However, the PCR technique enabled us to analyze the specific mutations in the Ki-*ras* genes.

DISCUSSION

In the present study, we detected a high frequency of activated Ki-*ras* oncogenes in both spontaneously occurring and chemically induced lung tumors of strain A mice. An activated Ki-*ras* oncogene was found in 91% of spontaneous tumors, 87% of B[*a*]P-induced tumors, 91% of EC-induced tumors, and 100% of MNU-induced tumors. The detection of the activated Ki-*ras* gene in lung adenomas, some <1 mm in diameter, suggests that the activation of this gene may be an early event in the chemical induction of lung tumors in strain A mice. A homogeneous cell population was seen during histopathological examinations of these adenomas. Sequence analysis of PCR-amplified exons of the Ki-*ras* gene from tumor DNA showed that the mutated allele and normal allele were present in approximately equal amount, suggesting that the mutated allele was present in most of the tumor cells. These data suggest that the activated Ki-*ras* gene was present in the cell whose clonal expansion gave rise to the adenoma. We have shown that, in the development of chemically induced lung adenocarcinomas in the strain A mouse, some adenocarcinomas are developed from the progression of hyperplastic lesion to adenoma to adenocarcinoma‡ arising within adenoma and then to adenocarcinoma (R.R.M., G.D.S., and M.W.A., unpublished data). Activated *ras* genes have been observed in several types of benign rodent tumors (6, 19, 20) as well as benign human colon tumors (21). It is possible that the activation of the Ki-*ras* protooncogene is the initiation event. In any case, activation of the Ki-*ras* gene is one step in the development of strain A mouse lung tumors.

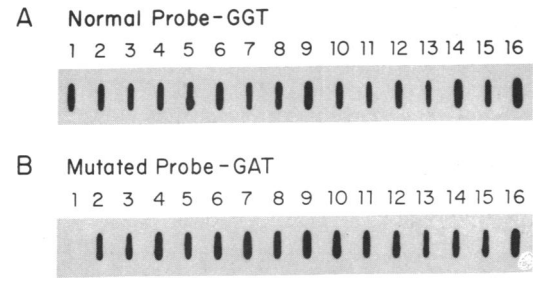

FIG. 3. Slot-blot analysis for codon 12 mutations. Amplified DNA samples were applied to duplicate nylon filters and hybridized with following probes: for wild-type codon 12, TTGGAGCTGGTGGCGTAGG (*A*) and for mutant codon 12, TTGGAGCTGATGGCGTAGG (*B*). Lanes: 1, amplified DNA sample from normal lung tissue of strain A mice; 2–16, various amplified DNA samples from MNU-induced lung tumors of strain A mice.

This study demonstrates *ras* protooncogene activation in B[*a*]P-induced rodent tumors. The G·C → T·A mutations (9/14 tumors) and G·C → A·T mutations (4/14 tumors) observed in B[*a*]P-induced lung tumors at codon 12 of the Ki-*ras* gene have also been detected in several *in vitro* systems (22–25). The presumed ultimate carcinogenic metabolite of B[*a*]P, [*r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-1,8,9,10-tetrahydrobenzo[*a*]pyrene (anti-B[*a*]PDE)], binds to guanosine at the N^2 position in native DNA and also forms lesser amounts of adducts with deoxyadenosine (26, 27). The anti-B[*a*]PDE induced predominantly G·C → T·A transversions in the *lacI* gene of *Escherichia coli* and the *APRT* gene in Chinese hamster ovary cells (22, 23). The G·C → A·T transitions were also detected although at a lower frequency. Stevens *et al.* (24) detected an activated human homologue of Ha-*ras* with a G·C → T·A transversion in a human fibroblast cell line transformed by anti-B[*a*]PDE. Another report has shown that the human homologue of Ha-*ras* can be activated by mutations at codons 12 and 61 after *in vitro* modification of the cloned DNA with anti-B[*a*]PDE (25). The point mutations induced by anti-B[*a*]PDE were predominantly G·C → T·A and A·T → T·A base substitutions (25). Our *in vivo* results on the activating mutations in the Ki-*ras* gene detected in B[*a*]P-induced lung tumors of strain A mice have substantiated the previous findings in *in vitro* model systems.

EC is carcinogenic in several rodent model systems. In this study, we have detected specific activation of Ki-*ras* protooncogenes in codon 61 by EC with 7 of 9 tumors having an A·T → T·A transversion and 2 of 9 tumors having an A·T →

‡Carcinoma arising within adenoma is characterized by focal areas of cytologic atypia, focal altered growth pattern, focal tinctorial differences, or a focal area (equivalent to more than three alveoli) where proliferating cell nuclei are crowded or piled up within a lesion that would otherwise be categorized as an adenoma.

G·C transition. A very similar spectrum of mutations (11/14 tumors, A·T → T·A transversions, and 2/14 tumors, A·T → G·C transitions) were observed in the Ha-*ras* oncogene detected in hepatomas of male B6C3F1 mice treated with EC and vinyl carbamate, a potential metabolite of EC (8, 28). Also, T. Bowden (personal communication) detected an activated Ha-*ras* protooncogene with an A·T → T·A transversion at the second base of codon 61 in papillomas (5/5 tumors) and carcinomas (3/3 tumors) induced by EC in mouse skin. The A·T → T·A transversions and A·T → G·C transitions are consistent with miscoding from deoxyadenosine adducts (29, 30). However, the major DNA adduct formed from EC and vinyl carbamate is likely a 7-(2-oxoethyl)guanosine adduct rather than deoxyadenosine adducts (31). Although it is clear that EC induced specific mutations in *ras* protooncogenes in several rodent tumor types, the mechanism(s) for the induction of these mutations by EC is unknown at present.

MNU is a very potent alkylating agent that preferentially modifies deoxyguanosine residues by methylating their N-7 and O^6 positions (32). As a consequence, modified deoxyguanosine residues may pair with thymidine instead of deoxycytosine, thus leading to the generation of G·C → A·T transitions. This specific mutation has been consistently observed in mutagenesis studies in both the *lacI* gene and *gpt* gene in *E. coli* (33, 34) and in Epstein–Barr virus shuttle vector in human cells (35). Activated Ha-*ras* genes have been identified in the majority of rat mammary tumors induced with a single dose of MNU during sexual development (15, 16). MNU activated the Ha-*ras* gene by a G·C → A·T transition at the second position of codon 12 in 61 of 61 tumors harboring NIH 3T3-transforming DNA (15, 16). In the present study, the same mutation, a G·C → A·T transition at the second position of codon 12, was observed in the Ki-*ras* gene in all (15/15) of the MNU-induced strain A mouse lung tumors examined. These observations indicated that the majority of the activating point mutations in the Ki-*ras* genes of MNU-, B[*a*]P-, and EC-induced lung tumors result from a direct genotoxic effect of these chemicals. The strain A mouse lung tumor model appears to be a very sensitive system for detecting the ability of chemicals to activate the Ki-*ras* protooncogene in lung tissue *in vivo*.

Activated Ki-*ras* protooncogenes have previously been detected in chemically induced rodent lung tumors and in human pulmonary adenocarcinomas. In tetranitromethane-induced rat lung tumors, an activated Ki-*ras* gene with a G·C → A·T transition at the second position of codon 12 was observed in 19 of 20 tumors (20). The same mutation in the Ki-*ras* gene was found in 10 of 10 tetranitromethane-induced B6C3F1 mouse lung tumors (20). Rodenhuis *et al.* (36) and our laboratory (S. Reynolds and M.W.A., unpublished data) detected an activated Ki-*ras* gene in ≈40% of human pulmonary adenocarcinomas examined. These data together with our present findings suggest that activation of the Ki-*ras* protooncogene is involved in the induction of pulmonary adenocarcinomas in both rodents and humans. The occurrence of a high percentage of activated Ki-*ras* genes in human pulmonary adenocarcinomas provides strong credibility to the strain A mouse as a model to study the development of this tumor type.

We thank Dr. Bengt Widergren for his help in the development of the PCR technique, Dr. Ken Tindall for assistance with direct sequencing, and Ms. Mary Morgan for the collection of mouse lung tissues. We also thank Drs. Steven Reynolds, Roger Wiseman, and Carl Barrett for the critical reading of this manuscript.

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