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Parental bias of Ki-*ras* oncogenes detected in lung tumors from mouse hybrids

(carcinogenesis/genetic susceptibility)

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ABSTRACT **A mouse strain with low lung tumor susceptibility (C3H) and a strain with high lung tumor susceptibility (A/J) were reciprocally crossed to produce C3A and AC3 F_1 hybrid mice. Ki-*ras* oncogenes were detected in spontaneous and chemically induced lung tumors obtained from the C3A and AC3 mice. To further explore the genetics of the Ki-*ras* gene in mouse lung tumor susceptibility, the parental origin of Ki-*ras* oncogenes detected in lung tumors from the F_1 hybrids was determined by a strategy based on a 37-base-pair deletion in the second intron of the A/J Ki-*ras* allele. Ki-*ras* oncogenes were derived from the A/J parent in 38 of 40 tumors obtained from C3A mice and 30 of 30 tumors from AC3 mice. The observation that the activated oncogene in hybrids originates from the susceptible parent suggests that the Ki-*ras* gene is directly linked to mouse lung tumor susceptibility. This finding may have implications for pulmonary adenocarcinoma development in humans, since Ki-*ras* oncogenes are detected in 35% of this human tumor type.**

Inbred strains of mice vary markedly in their susceptibility to spontaneous and chemically induced lung tumors (1). The A/J mouse is one of the most sensitive strains, whereas the C3H mouse is among the strains resistant to lung tumor development (2–4). A 90% spontaneous tumor incidence at 18 months of age is observed in A/J mice versus a 5–10% incidence in the C3H strain (2). A single dose of urethane (300 mg/kg) induced 7.2 tumors per mouse after 24 weeks in the A/J strain versus 0.2 tumor per mouse in the C3H strain, and similar differences were detected at lower doses of the carcinogen (4). Analysis of lung tumorigenesis in recombinant inbred mouse strains suggests that three genetic loci contribute to the observed differences in susceptibility to lung tumor development. Malkinson *et al.* (5, 6) have called these loci "pulmonary adenoma susceptibility" (*Pas*) genes. Statistical analysis of tumor data from such recombinant mice suggests that one of the *Pas* genes is a major contributor to susceptibility whereas the other two *Pas* genes are minor contributors. A histocompatibility locus and a gene that influences the proliferative rate of lung tumor progenitor cells are possible candidates for the *Pas* genes (5, 6) and the Ki-*ras* gene may be one of the minor *Pas* genes (2); however, no definitive identification of the *Pas* genes has been made.

The possible involvement of the Ki-*ras* gene in mouse lung tumor susceptibility is based on two observations. (*i*) The Ki-*ras* gene can be used as a genetic marker for lung tumor susceptibility in inbred strains of mice (2). Restriction fragment length polymorphism (RFLP) studies of the Ki-*ras* gene revealed a 0.70-kilobase (kb) *Eco*RI fragment in or near the first exon in lung tumor-resistant mouse strains and a slightly smaller fragment in susceptible strains (2). (*ii*) Spontaneously occurring and chemically induced lung tumors from the A/J mouse were found to have a high frequency (88–100%) of activated Ki-*ras* protooncogenes (7, 8). Ki-*ras* oncogenes have also been detected in spontaneous and chemically induced lung tumors from C3H mice; the tumors induced in C3H mice after repeated carcinogen administration, however, were smaller in size at the time of sacrifice, the tumor multiplicity was lower, and the latency period for tumor development was much longer than in the sensitive A/J mice (9). These data suggest that spontaneous and chemically induced lung tumors from the C3H × A/J (C3A) and A/J × C3H (AC3) F_1 hybrids should contain Ki-*ras* oncogenes. To further understand the genetics of the Ki-*ras* gene in mouse lung tumor susceptibility, we examined the parental origin of the activated Ki-*ras* protooncogenes detected in lung tumors from C3A and AC3 hybrid mice.

MATERIALS AND METHODS

Lung Tumor Induction. The C3H/HeJ and A/J parents of the C3A and AC3 F_1 hybrids originated from mice purchased from The Jackson Laboratory. Spontaneous lung tumors were collected from untreated 2-year-old C3A mice. Six- to 8-week-old C3A or AC3 mice were given i.p. injections of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK, 50 mg/kg), dissolved in phosphate-buffered saline, three times per week for 8 weeks. Controls were given i.p. injections of saline solution. Vinyl carbamate (VC) was administered to 7-week-old C3A or AC3 mice in a single injection (either 60 or 20 mg/kg). Tumors were harvested from chemically treated animals between 6 and 14 months of age. This time frame of tumor collection enabled us to obtain both adenomas and adenocarcinomas. Lung tumors were collected, frozen in liquid nitrogen, and then stored at −80°C. Before freezing, a representative portion of each tumor was fixed in neutral buffered 10% formalin for histopathological examination.

Polymerase Chain Reaction (PCR). The primer-directed enzymatic amplification of specific Ki-*ras* DNA sequences was carried out as described (10) with *Taq* DNA polymerase. Reactions were carried out in a 100-μl mixture that contained 2 μg of DNA, 50 mM KCl, 10 mM Tris (pH 8.4), 2.5 mM $MgCl_2$, 200 μM dNTPs, gelatin at 200 μg/ml, 2 units of *Taq* DNA polymerase (Perkin–Elmer/Cetus), and two primers. Samples were subjected to 25 cycles of amplification in a thermal cycler (DNA denaturation at 94°C for 1 min, primer annealing at 45°C for 2 min, and extension at 72°C for 2 min). The double-stranded DNA created in PCR was purified and concentrated from 100 μl to 40 μl by using Centricon-30 microconcentrators (Amicon). The primers used to amplify the first exon of Ki-*ras* were 5′-ATGACTGAGTATAAACT-

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Abbreviations: RFLP, restriction fragment length polymorphism; NNK, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone; VC, vinyl carbamate.

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TGT-3′ and 5′-CTCTATCGTAGGGTCGTACT-3′, and those for the second exon were 5′-ACTCCTACAGGAAACAAGT-3′ and 5′-CTATAATGGTGAATATCTTC-3′. The primer pairs corresponded in each case to the first and last 20 bases of the exon according to the published Ki-*ras* gene sequence (11). Other amplification primers, based on the sequence in Fig. 2, are described in the appropriate figure legends and the primers used in the inverse PCR procedures are described in the next section. All primers were synthesized on an Applied Biosystems 380B DNA synthesizer (Applied Biosystems).

Inverse PCR. The inverse PCR procedure (12, 13) was used to amplify the 0.7-kb *Eco*RI Ki-*ras* fragment of C3H mouse genomic DNA and the corresponding slightly smaller fragment from A/J mouse genomic DNA. DNAs isolated from both the C3H and A/J mouse lung tissues were digested with *Eco*RI endonuclease and then circularized by T4 DNA ligase. The circularized products were randomly linearized by heating at 95°C for 15 min and then amplified, as described in the previous PCR section, by using primers synthesized in the opposite orientations to primers used in amplifying the first exon of Ki-*ras*. The choice of the primers is based on the observation that the first exon of Ki-*ras* is contained within the 0.7-kb *Eco*RI fragment of mouse Ki-*ras*. The primers used were: 5′-CTTTGTGGATGAGTACGACCCTACG-3′ (sense) and 5′-TGTATCGTCAAGGCGCTCTTGCCTACGGCA-3′ (antisense).

Direct Sequencing of PCR-Amplified DNA. Samples (0.5 μg) of amplified DNAs were directly sequenced by the dideoxy method (14). Sequencing primers were end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (Pharmacia). Sequencing primers were 5′-AGGTGATTCTGAATTAGCTG-3′ (antisense) for the first exon of Ki-*ras* and 5′-AATTGATGGAGAAACCTGTC-3′ (sense) for the second exon. The following sequencing primers were used to sequence the *Eco*RI Ki-*ras* fragment amplified by inverse PCR: primers for sequencing the sense strand were sense PCR primer (described under *Inverse PCR*), 5′-AATTATATTGAAAGTTATTT-3′, and 5′-TTGTGAGAGTTAAGAATATA-3′; primers for sequencing the antisense strand were antisense PCR primer (described under *Inverse PCR*), 5′-GGCTGCATAGTAAGACCCTG-3′, and 5′-GCACCTATGGTTCCCTAACA-3′.

Transfection Assay. High molecular weight DNA from normal and tumor tissues was transfected into NIH 3T3 mouse fibroblasts (30 μg of DNA per plate; four plates per sample) by the calcium phosphate precipitation method (15). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO) containing 5% calf serum (Colorado Serum, Denver). Focus formation was scored at days 17–28. Focus cells were isolated and mass cultured in DMEM/5% calf serum for DNA isolation. The DNAs were analyzed by PCR and direct sequencing as described above.

RESULTS

Tumor Response. Spontaneous lung tumors were found in ≈50% of vehicle-treated or untreated C3A mice. Over 90% of the NNK- or VC-treated C3A or AC3 mice had lung tumors. Lung tumor latency was also reduced in chemically treated mice. A high incidence of tumors were observed in chemically treated mice between 6 and 9 months of age, whereas lung tumors were rarely observed in control mice <18 months of age. The tumors were diagnosed as either adenomas or papillary adenocarcinomas.

The tumorigenic response of the hybrids appears to be between that of the parents. A single 60-mg/kg dose of VC induced 34 tumors per mouse in the A/J strain, 12 tumors per mouse in the C3A hybrid, 8 tumors per mouse in the AC3 hybrid, and <1 tumor per mouse in the C3H strain when mice were sacrificed at 8–10 months of age. The data for the C3H strain are from ref. 4 and the data for the other strains consist of unpublished observations from our laboratory.

Identification of Activating Mutations in Ki-*ras*. Mouse lung tumors obtained from the C3A and AC3 F_1 hybrids were analyzed for Ki-*ras* oncogenes by direct sequencing of PCR-amplified tumor DNAs. Sequence analysis was performed on the first exon (spans region containing codons 12 and 13) and the second exon (spans region containing codon 61) of the Ki-*ras* oncogene. Representative autoradiograms of sequence analysis are shown in Fig. 1. Activating point mutations in codons 12 or 61 of the Ki-*ras* gene were observed in 20 of 21 of the spontaneous tumor DNAs, 7 of 7 of the NNK-induced tumor DNAs, and 13 of 14 of the VC-induced tumor DNAs from the C3A hybrid and in 19 of 20 of the NNK-induced tumor DNAs and 11 of 12 of the VC-induced tumor DNAs from the AC3 hybrid.

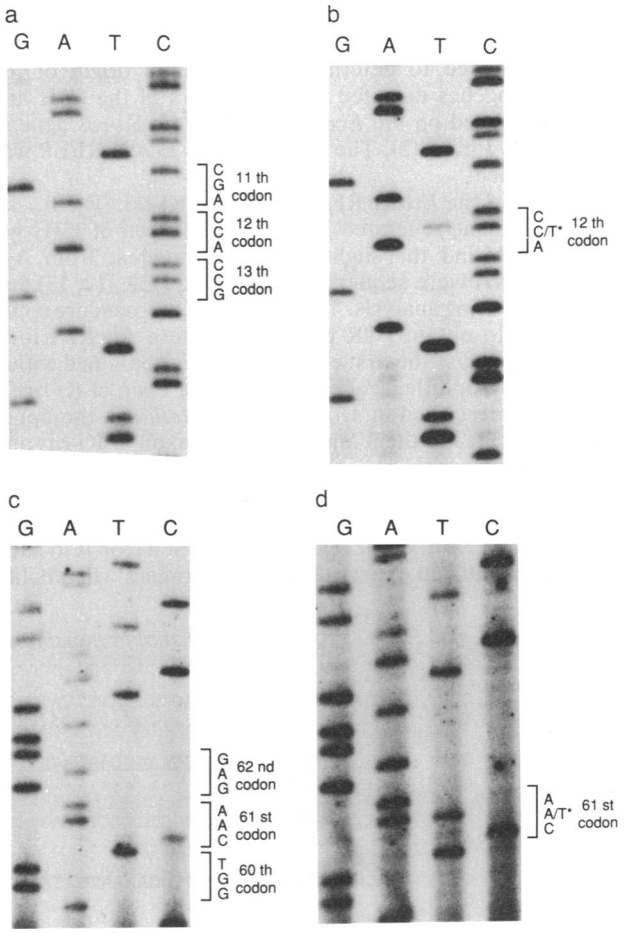

FIG. 1. Identification of mutations in codons 12 and 61 of the Ki-*ras* gene by direct sequencing of PCR-amplified tumor DNAs. Representative autoradiogram of sequence analysis is shown. The Ki-*ras* first exon and second exon were PCR-amplified and directly sequenced as described in *Materials and Methods*. Lanes: G, guanine; A, adenine; T, thymine; C, cytosine. (*a*) Normal sequence of codons 11–13 of Ki-*ras* from AC3 mouse genomic DNA. Note that the sequence of the antisense strand is shown for exon 1. (*b*) Detection of a G·C → A·T transition at second position of codon 12 in DNA from an AC3 pulmonary adenoma induced by NNK. Both the mutated and the normal allele were observed in the lung tumor DNA. (*c*) Normal sequence of codons 60–62 of Ki-*ras* from C3A mouse genomic DNA. The sense-strand sequence is shown for exon 2. (*d*) Detection of an A·T → T·A transversion at second position of codon 61 in DNA from a C3A pulmonary adenocarcinoma induced by VC. Both the mutated and the normal allele were observed in the lung tumor DNA.

Table 1. Mutation spectrum in Ki-*ras* oncogenes detected in lung tumors from C3A or AC3 mice

Lung tumors	No. of tumors with Ki-*ras* oncogenes	No. of activating mutations					
		Codon 12 (GGT)				Codon 61 (CAA)	
		TGT	GTT	GAT	CGT	CTA	CGA
Spontaneous C3A	20	1	1	4	1	4	9
NNK-induced C3A	7	0	0	7	0	0	0
VC-induced C3A	13	0	0	0	0	7	6
NNK-induced AC3	19	0	0	19	0	0	0
VC-induced AC3	11	0	0	0	0	6	5

Tumors were either adenomas or adenocarcinomas. Normal sequences of codons 12 and 61 are shown in parentheses.

Activating mutations in the C3A and AC3 lung tumors are presented in Table 1. These mutation patterns are similar to those previously observed in spontaneous and NNK- or VC-induced lung tumors from A/J (refs. 7–9 and unpublished data).

We proceeded to determine the parental origin of the Ki-*ras* oncogenes detected in lung tumors of the C3H and AC3 mice based on the *Eco*RI RFLP in the Ki-*ras* gene in C3A and A/J mice (2). The nature of this *Eco*RI RFLP was first examined.

Analysis of the *Eco*RI RFLP in Mouse Ki-*ras*. The 0.7-kb Ki-*ras* fragment generated by *Eco*RI digestion of C3H genomic DNA and the slightly smaller fragment from A/J genomic DNA were sequenced by inverse PCR (12, 13) and direct sequencing analysis. The inverse PCR procedure could be utilized to obtain PCR products of these *Eco*RI Ki-*ras* fragments because the first exon of Ki-*ras* is contained within the fragments and the sequence of the first exon of Ki-*ras* is known. As described in *Materials and Methods*, the upper primer is located at the 3′ end of the first exon of Ki-*ras* and will amplify toward the second intron and the lower primer is located at the 5′ end of the first exon of Ki-*ras* and will amplify into the first intron. There is a 5′ untranslated exon (exon 0) in the Ki-*ras* gene, and thus the first intron is located between exon 0 and exon 1 (16). These primers will amplify the circularized *Eco*RI fragments that are randomly linearized in the middle of the first exon of Ki-*ras*. Thus, the portion of the sequence of the first and second introns that is included in the *Eco*RI fragment can be determined from this PCR product. The PCR products were then directly sequenced (Fig. 2). The sizes of the *Eco*RI fragments are 714 base pairs (bp) and 677 bp in the C3H and A/J genome, respectively. Thus, the observed *Eco*RI RFLP resulted from a 37-bp deletion in the second intron of the Ki-*ras* gene from the A/J mouse genome. Nucleotides 320–356 were deleted in the A/J genome (Fig. 2). The 37-bp difference in the two fragments is significantly smaller than the 150-bp difference suggested by Ryan *et al.* (2); however, in the *Eco*RI Southern blots shown by Ryan *et al.* (figures 1 and 2 in ref. 2), the Ki-*ras* fragment difference between the A/J and C3H Ki-*ras* is <100 bp, and our Southern blot showed a difference of <50 bp (data not shown).

Identification of Parental Origin of Ki-*ras* Oncogenes Detected in C3A and AC3 Mouse Lung Tumors. Several procedures based on the 37-bp deletion in the second intron of the A/J Ki-*ras* allele were employed to determine the parental origin of the Ki-*ras* oncogenes detected in the C3A and AC3 mouse lung tumors. First, DNAs from the C3A and AC3 tumors that contained mutations in codon 12 of the Ki-*ras* gene were amplified with PCR primers that flanked the 37-bp deletion. A representative agarose gel analysis of the PCR fragments is shown in Fig. 3*a*. The 483-bp and 520-bp fragments amplified from A/J and C3H genomic DNAs are shown in lanes 1 and 2, respectively. The two fragments observed in lane 3 are from normal C3A (or AC3) lung DNA, and lanes 4–9 represent AC3 or C3A lung tumor DNAs. The 520-bp band is from the C3H Ki-*ras* allele and the 483-bp band is from the A/J Ki-*ras* allele. Each of the two fragments was isolated and sequenced to determine which allele had the activating mutation in the 12th codon. In a representative autoradiogram of the sequence analysis (Fig. 3*b*), a G → A mutation in the second base of codon 12 is observed in the 483-bp A/J fragment, whereas only the normal Ki-*ras* sequence is observed in the 520-bp C3H fragment. The normal allele of Ki-*ras* is also observed in the sequence analysis of

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        -131
C3H       AATTCGGAATATCTTAGAGTTTTACACACAAAGGTGAGTGTTAAAACATTGATAAAGTTTTTGATAATCTTGTGTGA
A/J       ..........................................................................
        -54                                                                  1
C3H       GACATGTTCTAATTTAGTTGTATTTTATTATTTTTATTGTAAGGCCTGCTGAAAATGACTGAGTATAAACTTGTGGT
A/J       ..........................................................................
        24
C3H       GGTTGGAGCTGGTGGCGTAGGCAAGAGCGCCTTGACGATACAGCTAATTCAGAATCACTTTGTGGATGAGTACGACC
A/J       ..........................................................................
        101
C3H       CTACGATAGAGGTAACGCTGCTCTACAGTCTGCGTGCGCTTGTAAAGGACGGCAGCCAGCCGCTTTGAAAAAGATAT
A/J       ..........................................................................
        178
C3H       CGATTTTTATATTTATTAGAAAATTATATTGAAAGTTATTTCAGTTATATGTGATGTCCTTTAGTTCCAAGGCTTTA
A/J       ..........................................................................
        255                                   288      296                        320
C3H       AACTGGGTGTTAGGGAACCATAGGTGCAAGAAAGTCCACTTCTCATGAGAGCTCACCACAGAGAAAGAAAGTCCACT
A/J       ..............................C.......A.......................
        332                           356
C3H       TCTCATGAGAGCTTACCACAGAGAGTAAGGTCCAAGATGCCATCCGTGCTTTGTATTTGCATTTTGATAAATAAATT
A/J                                   ..................................................
        409
C3H       TCATGATTTTGTGAGAGTTAAGAATATAGCTTTGCCTTTGATCTATTCCTGAAAGGTTTGTTGTTGTTTTATGGGTT
A/J       ..........................................................................
        486     494
C3H       TGTTGTTATTGTGTTGTTGTTTGGTGTCTTTTAGGCAGGGTCTTACTATGCAGCCCTGACCGGCCTAGAACTCAACT
A/J       ........C.................................................................
        563
C3H       ATGTAGACCAGACTAGCTCTG
A/J       .....................
```

FIG. 2. Sequence analysis of the 714-bp (C3H allele) and the 677-bp (A/J allele) *Eco*RI fragments of Ki-*ras*. The *Eco*RI fragments of Ki-*ras* were amplified by inverse PCR and then directly sequenced. The sequence of the C3H *Eco*RI fragment of Ki-*ras* is 714 bp and includes 131 bases of the first intron directly upstream of the translation start codon, the 37 translated codons of the first exon, and 472 bases of the second intron. The first nucleotide of the translation start codon is designated 1. The A/J fragment is 37 bp shorter and the deleted region in the second intron included bases 320–356. Three other base changes in the second intron were observed between the A/J and C3H fragments.

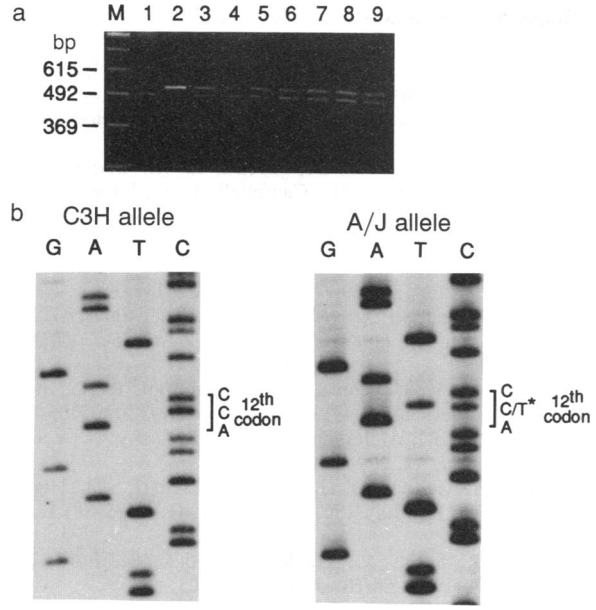

FIG. 3. PCR and direct sequencing analysis to determine the parental origin of Ki-*ras* oncogenes with codon 12 mutations detected in AC3 or C3A mouse lung tumors. (*a*) DNAs isolated from lung tumors that contained Ki-*ras* oncogenes with codon 12 mutations and normal lung tissues were subjected to PCR with primers that flanked the 37-bp deletion in the second intron of the A/J Ki-*ras* allele: primer I, bases 1–20 in Fig. 2; primer II, complementary to bases 506–520 in Fig. 2. Lanes: 1, A/J DNA; 2, C3H DNA; 3, AC3 DNA; 4–6, NNK-induced AC3 lung tumor DNAs; 7, C3A spontaneous lung tumor DNA; 8 and 9, NNK-induced C3A lung tumor DNAs. (*b*) The two amplified fragments in lanes 4–9 were individually isolated and then directly sequenced. A representative autoradiogram of sequence analysis is shown for the fragments in lane 5 of *a*. A G·C → T·A transversion in the second base of codon 12 of Ki-*ras* was observed in the 483-bp A/J fragment, whereas no mutation was observed in the 520-bp C3H fragment. These fragments were generated from the tumor DNA presented in Fig. 1*b*. Both the mutated and normal alleles were observed in the 483-bp A/J allele.

the A/J fragment. This probably results from contamination of tumor cell DNA with DNA from normal cells.

Sequence analysis showed that 32 of the tumors contained a codon 12 mutation in the 483-bp fragment from the A/J allele, and one tumor (a spontaneous tumor from a C3A mouse) had a mutation in the 520-bp fragment from the C3H allele. Thus 32 of 33 of the Ki-*ras* oncogenes detected in C3A or AC3 tumors that had a codon 12 mutation were derived from the A/J parent and 1 of 33 from the C3H parent.

We next analyzed the tumors that contained mutations in codon 61 of Ki-*ras*. PCR fragments that contained both codon 61 and the 37-bp region in intron 2 were too large to separate and individually sequence, as was the case for the codon 12 mutants (Fig. 3). The oncogenic Ki-*ras* alleles in the tumors that contained a codon 61 mutation were isolated by the NIH 3T3 transfection assay. DNAs isolated from the lung tumors that contained Ki-*ras* oncogenes with codon 61 mutations exhibited transforming activity in the NIH 3T3 focus assay. DNAs from representative foci from each of the tumor samples tested contained a Ki-*ras* gene with a codon 61 mutation identical to the tumor DNA. The allelic identity of these Ki-*ras* oncogenes was determined by PCR analysis of the DNAs isolated from the foci. A representative agarose gel analysis of the PCR fragments is shown in Fig. 4. The amplification primers used in Fig. 3*a* were also employed in the PCR analysis shown in Fig. 4. Amplified foci DNA with these primers will generate both the 520-bp C3H fragment (Fig. 4, lane 2) and the 483-bp NIH 3T3 fragment (lane 13) if the Ki-*ras* oncogene is from the C3H parent. In contrast, only

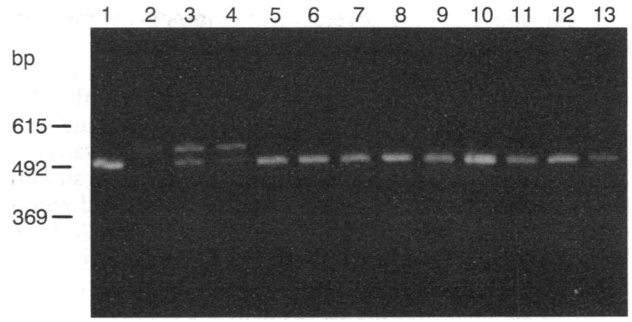

FIG. 4. PCR analysis of NIH 3T3 foci DNAs to determine the parental origin of Ki-*ras* oncogenes with codon 61 mutations detected in AC3 or C3A mouse lung tumors. Morphologically transformed NIH 3T3 foci were derived from AC3 or C3A mouse lung tumor DNAs that contained Ki-*ras* oncogenes with codon 61 mutations. The NIH 3T3 foci were induced by the Ki-*ras* oncogenes. The amplification primers were primers I and II defined in the legend of Fig. 3. A 483-bp fragment of Ki-*ras* was generated from A/J mouse and NIH 3T3 cell DNAs, and a 520-bp fragment from C3H mouse DNA. Ethidium bromide staining of PCR products separated in a 1.5% agarose gel is shown. Lane 1, A/J mouse lung DNA; lane 2, C3H mouse lung DNA; lane 3, C3A mouse lung DNA; lanes 4–7, DNAs of NIH 3T3 foci derived from spontaneous C3A mouse lung tumors; lanes 8–10, DNAs of NIH 3T3 foci derived from VC-induced C3A mouse lung tumors; lanes 11 and 12, DNAs of NIH 3T3 foci derived from VC-induced AC3 mouse lung tumors; lane 13, NIH 3T3 DNA.

the 483-bp fragment will be generated if the Ki-*ras* oncogene is derived from the A/J allele (lane 1). Only one focus DNA exhibited both the 520-bp and 483-bp PCR fragments (lane 4); thus, the transforming Ki-*ras* gene was derived from the C3H parent in this spontaneous C3A lung tumor. The other 8 foci DNA samples (lanes 5–12) generated only the 483-bp PCR fragment; thus, the transforming Ki-*ras* genes in these C3A or AC3 tumors were derived from the A/J parent. This analysis showed that 36 of 37 of the Ki-*ras* oncogenes detected in C3A or AC3 tumors with a codon 61 Ki-*ras* mutation were derived from the A/J allele and 1 of 37 from the C3H allele. Table 2 summarizes the parental origin of Ki-*ras* oncogenes detected in C3A or AC3 mouse lung tumors.

DISCUSSION

We report the almost exclusive localization of an activated protooncogene to a parental-specific allele in tumors obtained from hybrid animals. This observation is different from the example of genome imprinting that has been seen in several types of human pediatric tumors. A tumor-suppressor gene, the Wilms tumor gene, was inactivated exclusively in the father during spermatogenesis (17–20). In the present study, the Ki-*ras* allele of the A/J mouse was activated to an

Table 2. Parental origin of Ki-*ras* oncogenes detected in C3A or AC3 mouse lung tumors

F_1 hybrid	Treatment	No. of samples with Ki-*ras* oncogenes	Parental origin of Ki-*ras* oncogenes	
			A/J allele	C3H allele
C3A	None	20	18	2
	NNK	7	7	0
	VC	13	13	0
AC3	NNK	19	19	0
	VC	11	11	0

oncogene and led to the development of tumors independent of the sex of the A/J mouse in F_1 hybrids.

Since the activated Ki-*ras* allele detected in the tumors from the C3A and AC3 hybrids originates from the A/J parent, it is very possible that the Ki-*ras* gene could be a susceptibility locus for mouse lung tumorigenesis. Ryan *et al.* (2) suggested that the Ki-*ras* locus may be a minor susceptibility locus, on the basis of RFLP analysis and tumor incidence in recombinant hybrids generated from A/J and C57BL/6J mice. The C57BL/6J inbred mouse is resistant to lung tumor induction, similar to the C3H mouse, and the *Eco*RI RFLP bands for the Ki-*ras* gene are identical in these two mouse strains (2). Ryan *et al.* (2) did not analyze protooncogene activation in the recombinant inbreds. Oncogene analysis in the recombinant inbreds derived from the A/J and C3H mice and the A/J and C57BL/6J mice could further our understanding of susceptibility loci.

Several mechanisms for the preferential activation of the Ki-*ras* gene from the A/J parent can be postulated. The A/J allele could be more prone to mutations in regions such as codons 12 or 61, which activate the Ki-*ras* protooncogene, than the allele from the C3H parent. Recent studies in our laboratory suggest that the difference in lung tumor induction between the C3H and A/J mice by methylating agents is not due to O^6-methylguanine DNA adduct levels (9). NNK-induced mouse lung tumors in the C3H mouse contain the same high frequency of activated Ki-*ras* genes as observed in the A/J mouse under conditions of similar initial adduct levels. However, the C3H tumor multiplicity is less, the size of the tumors are smaller, and the latency period for tumor development is longer. Also, activated Ki-*ras* genes were detected at a much lower frequency (6/17) in spontaneous C3H lung tumors than in spontaneous A/J (19/20) or C3A (20/21) lung tumors (refs. 7 and 9; unpublished data). These previous results, coupled with the present findings, suggest an enhanced expression of the A/J allele of Ki-*ras* in the F_1 hybrids. Enhanced expression of one activated Ki-*ras* allele (A/J) relative to the other (C3H) could lead to a more rapid clonal expansion of a lung cell and subsequently the appearance of a tumor in the F_1 hybrid that contains an activated Ki-*ras* gene in the A/J allele. Differences in the DNA sequence of promoter/enhancer regions of the A/J and C3H Ki-*ras* gene could lead to preferential gene expression (21, 22). Ki-*ras* oncogenes have been detected in numerous human tumors (23, 24), including 35% of pulmonary adenocarcinomas from smokers (25, 26). If a Ki-*ras* allele itself can confer tumor susceptibility in a rodent, then similar types of alleles might exist in humans and be involved in the development of some human pulmonary adenocarcinomas, and perhaps also in other tumor types that contain Ki-*ras* oncogenes.

1. Shimkin, M. B. & Stoner, G. D. (1975) *Adv. Cancer Res.* **21**, 1–58.
2. Ryan, J., Barker, P. E., Nesbitt, M. N. & Ruddle, F. H. (1987) *J. Natl. Cancer Inst.* **79**, 1351–1357.
3. Malkinson, A. M. & Beer, D. S. (1983) *J. Natl. Cancer Inst.* **70**, 931–936.
4. Allen, J. W., Stoner, G. D., Pereira, M. A., Backer, L. C., Sharief, Y., Hatch, G. G., Campbell, J. A., Stead, A. G. & Nesnow, S. (1986) *Cancer Res.* **46**, 4911–4915.
5. Malkinson, A. M., Nesbitt, M. N. & Skamene, E. (1985) *J. Natl. Cancer Inst.* **75**, 971–974.
6. Malkinson, A. M. (1989) *Toxicology* **54**, 241–271.
7. You, M., Candrian, U., Maronpot, R. R., Stoner, G. D. & Anderson, M. W. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 3070–3074.
8. Belinsky, S. A., Devereux, T. R., Maronpot, R. R., Stoner, G. D. & Anderson, M. W. (1989) *Cancer Res.* **49**, 5305–5311.
9. Devereux, T. R., Anderson, M. W. & Belinsky, S. A. (1991) *Carcinogenesis* **12**, 299–303.
10. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–490.
11. George, D., Scott, A., Trusko, S., Glick, B., Ford, E. & Dorney, D. (1985) *EMBO J.* **4**, 1199–1203.
12. Ochman, H., Gerber, A. S. & Hartl, D. L. (1988) *Genetics* **120**, 621–623.
13. Triglia, T., Peterson, M. G. & Kemp, D. J. (1988) *Nucleic Acids Res.* **16**, 8186.
14. Tindall, K. & Stankowski, L. (1989) *Mutat. Res.* **220**, 241–253.
15. Reynolds, S. H., Stowers, S. J., Maronpot, R. R., Aaronson, S. & Anderson, M. W. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 33–37.
16. Orita, M., Sekiya, T. & Hayashi, K. (1990) *Genomics* **8**, 271–278.
17. Sapienza, C. (1990) *Mol. Carcinogen.* **3**, 118–121.
18. Scrable, H. J., Sapienza, C. & Cavenee, W. K. (1990) *Adv. Cancer Res.* **54**, 25–62.
19. Toguchida, J., Ishizaki, K., Sasaki, M. S., Nakamura, Y., Ikenaga, M., Katao, M., Sugimot, M., Kotoura, Y. & Yamamuro, T. (1989) *Nature (London)* **338**, 156–158.
20. Schroeder, W. T., Chao, L. Y., Dao, D. D., Strong, L. C., Pathak, S., Riccardi, V., Lewis, W. H. & Saunders, G. F. (1987) *Am. J. Hum. Genet.* **40**, 413–420.
21. Hoffman, E. K., Trusko, S. P., Freeman, N. A. & George, D. L. (1987) *Mol. Cell. Biol.* **7**, 2592–2596.
22. Hoffman, E. K., Trusko, S. P., Murphy, M. & George, D. L. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2705–2709.
23. Bos, J. L. (1989) *Cancer Res.* **49**, 4682–4689.
24. Barbarid, M. (1987) *Annu. Rev. Biochem.* **56**, 779–827.
25. Reynolds, S. H., Anna, C. K., Brown, K. C., Wiest, J. S., Beattie, E. J., Pero, R. W., Iglehart, J. D. & Anderson, M. W. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1085–1089.
26. Rodenhuis, S., Slebos, R., Boot, A., Evers, S., Mooi, W., Wagennar, S., van Bodegom, P. & Bos, J. (1988) *Cancer Res.* **48**, 5738–5741.