Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 1085–1089, February 1991
Biochemistry

Activated protooncogenes in human lung tumors from smokers

(carcinogenesis/point mutation/polymorphism/metastasis)

STEVEN H. REYNOLDS*†, COLLEEN K. ANNA*, KATHRYN C. BROWN*, JONATHAN S. WIEST*, EDWARD J. BEATTIE‡, RONALD W. PERO§, J. DIRK IGLEHART¶, AND MARSHALL W. ANDERSON*

*National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709; ‡Beth Israel Medical Center, New York, NY 10003; §New York University Medical Center, New York, NY 10016; and ¶Duke University Medical Center, Durham, NC 27710

Communicated by George H. Hitchings, November 5, 1990

ABSTRACT **Fourteen primary human lung tumor DNAs from smokers were analyzed for transforming activity by two DNA transfection assays. Activated protooncogenes were detected in 3 of 11 tumor DNAs by the NIH 3T3 focus assay, whereas activated protooncogenes were detected in 11 of 13 tumor DNAs by the NIH 3T3 cotransfection–nude mouse tumorigenicity assay. *K-* or *NRAS* genes activated by point mutation at codons 12 or 61 were detected in a large cell carcinoma, a squamous cell carcinoma, and 5 adenocarcinomas. An *HRAS* oncogene activated by a different mechanism was detected in an epidermoid carcinoma. One adenocarcinoma was found to contain an activated *RAF* gene. Two unidentified transforming genes were detected in a squamous cell carcinoma DNA and two adenocarcinoma DNAs. Eight of 10 lung adenocarcinomas that had formed metastases at the time of surgery were found to contain *RAS* oncogenes. No significant increase in metastasis was observed in the lung adenocarcinomas that contained one or more 6-kilobase *Eco*RI alleles of the *LMYC* gene. Overall, 12 of 14 (86%) of the lung tumor DNAs from smokers were found to contain activated protooncogenes. *RAS* oncogenes appear to play a role in the development of metastases in lung adenocarcinomas.**

Epidemiological studies have indicated that ≈85% of all lung cancer deaths in the United States are associated with tobacco smoking (1, 2). It is generally thought that an initial step in lung tumorigenesis in smokers involves the conversion of some of the 3800 chemicals in cigarette smoke (1, 3, 4) to electrophilic species that can react covalently with DNA to yield promutagenic DNA adducts. ^{32}P postlabeling techniques have shown that cigarette smokers have higher DNA adduct levels than nonsmokers and many of the DNA adducts appear to be smoking specific (5, 6). A causal relationship between specific chemicals and lung cancer has not been established, but a linear relationship has been established between the number of cigarettes smoked and lung cancer risk (7).

Two classes of genes, protooncogenes and tumor suppressor genes, are highly conserved in nature and therefore appear to be involved in normal cellular processes such as growth, differentiation, and regulation of gene expression (8, 9). Investigations in rodent models for chemical carcinogenesis imply that certain types of protooncogenes are activated by carcinogen treatment and that this activation process is an early event in tumor induction (10–13).

Lung tumors from smokers provide a unique opportunity to investigate protooncogene activation in a chemical-associated human tumor type. Polymerase chain reaction (PCR) gene amplification followed by oligonucleotide mismatch hybridization or single-strand conformation polymorphism analysis have detected activated *RAS* genes in approximately one-third of primary adenocarcinomas of the lung and at lower frequency in other forms of non-small-cell lung cancer (14–16). We have therefore used two general oncogene detection techniques, the NIH 3T3 focus assay and the NIH 3T3 cotransfection–nude mouse tumorigenicity assay, to analyze protooncogene activation in a series of primary human lung tumors from smokers. Our results provide insight into the mechanisms and frequency of protooncogene activation in chemical-associated human tumors.

MATERIALS AND METHODS

Source of Human Lung Tumors. Human lung tumors were obtained from Beth Israel Hospital and Duke University Medical Center.

Transfection Assays. High molecular weight DNA was isolated from normal or tumor tissues and DNA transfection analysis for the NIH 3T3 focus assay was performed as described (17).

Nude Mouse Tumorigenicity Assays. The nude mouse tumorigenicity assay was performed as described (18).

Southern Blot Analysis. Southern blot analysis was performed as described (17). The DNA hybridization probes were as described in the figure legends.

DNA Amplification. Amplification reactions were carried out as described (19).

Direct DNA Sequencing of Amplified DNA. Amplified *RAS* exons were sequenced by a modification of the dideoxynucleotide method (20). The sequencing primers were end-labeled with [^{32}P]ATP (Amersham; >5000 Ci/mmol; 1 Ci = 37 GBq) by the method of Zarbl *et al.* (12).

Slot-Blot Oligonucleotide Hybridizations. Amplified DNA samples (500 ng) were mixed with 100 μl of 0.4 M NaOH/25 mM EDTA and applied to Nytran nylon filters with a slot-blot apparatus. The filters were hybridized and washed according to the method of Verlaan-de Vries *et al.* (21).

RESULTS

Transforming Activity of DNA from Human Lung Tumors. Three of 11 tumor DNAs exhibited transforming activity in the NIH 3T3 focus assay, whereas 11 of 13 tumor DNAs exhibited transforming activity in the NIH 3T3 cotransfection–nude mouse tumorigenicity assay (Table 1). The tumors that arose in nude mice appeared with a latency of 21–77 days after injection of NIH 3T3 cells that had been transfected with human lung tumor DNAs. Transfection of DNA from the T24 bladder carcinoma cell line, which contains a codon 12-activated *HRAS* oncogene, gave tumors at eight of eight injection sites with a latency of 21–35 days. Control transfections using NIH 3T3 or normal human lung DNA typically gave one tumor per eight injection sites, with a latency of 63–112 days.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: PCR, polymerase chain reaction.

†To whom reprint requests should be addressed.

Table 1. Transforming genes in human lung tumors

DNA source	Focus assay	Tumorigenicity assay*	Latency, days	Transforming gene	Activating mutation
T24 cell line	+	8/8	21–35	*HRAS*	Codon 12
NIH 3T3 cells or normal human lung	−	1/8	63–112		
Large cell carcinoma 31	+	8/8	21–56	*KRAS*	Codon 12
Adenocarcinoma 1	+	NT		*KRAS*	Codon 12
Adenocarcinoma 14	−	5/16	35–70	c-*RAF*	
Adenocarcinoma 27	−	3/8	42–77		
Adenocarcinoma 32	+	11/12	21–56	*KRAS*	Codon 12
Adenocarcinoma 33	−	8/20	28–63	non-*RAS*	
Adenocarcinoma 36	−	5/8	21–91	*KRAS*	Codon 12
Adenocarcinoma 41	−	5/8	28–63	non-*RAS*	
Adenocarcinoma 43	NT	4/8	21–49	*KRAS*	Codon 12
Adenocarcinoma 46	NT	6/8	21–42	*NRAS*	Codon 61
Epidermoid carcinoma 3	−	1/8	35	*HRAS*	
Squamous cell carcinoma 11	−	0/8			
Squamous cell carcinoma 40	−	3/8	49–70	non-*RAS*	
Squamous cell carcinoma 81	NT	7/8	21–35	*NRAS*	Codon 12

NT, not tested.
*Tumors/injection sites.

Identification of Transforming Genes in Foci and Nude Mouse Tumor DNAs. The foci and nude mouse tumor DNAs were initially analyzed for the presence of human *Alu* repetitive DNA sequences (data not shown). Those DNAs that were *Alu* positive were then screened by Southern blot analysis with DNA probes specific for *H-*, *K-*, and *NRAS*, *RAF*, *MET*, *NEU*, *MYC*, *ABL*, and *ERBB*. We determined that five activated *KRAS* genes (Fig. 1*A*; Table 1), one activated *HRAS* gene (Fig. 1*B*; Table 1) and two activated *NRAS* genes (Fig. 1*C*; Table 1) were present in 8 of the 14 lung tumor DNAs analyzed.

Two primary nude mouse tumor DNAs derived from transfection of adenocarcinoma 14 DNA were found to contain human *RAF* genes (Table 1; Fig. 2, lanes e and h). Retransfection of these two primary nude mouse tumor DNAs resulted in tumor formation at eight of eight injection sites with a tumor latency of 21–28 days. Southern blot analysis of those secondary nude mouse tumor DNAs revealed that the process of tumorigenicity segregated with the presence of human *RAF* genes (lanes f, g, i, and j). A number of investigators have detected an artifactually activated *RAF* gene by DNA transfection techniques, but this type of activation typically occurs as a single event (22–24). The DNA restriction pattern of the two *RAF* containing primary nude mouse tumor DNAs (lanes e and h) demonstrates that these two tumors were the result of different transfection events and thus represent multiple detection of *RAF* from adenocarcinoma 14 DNA.

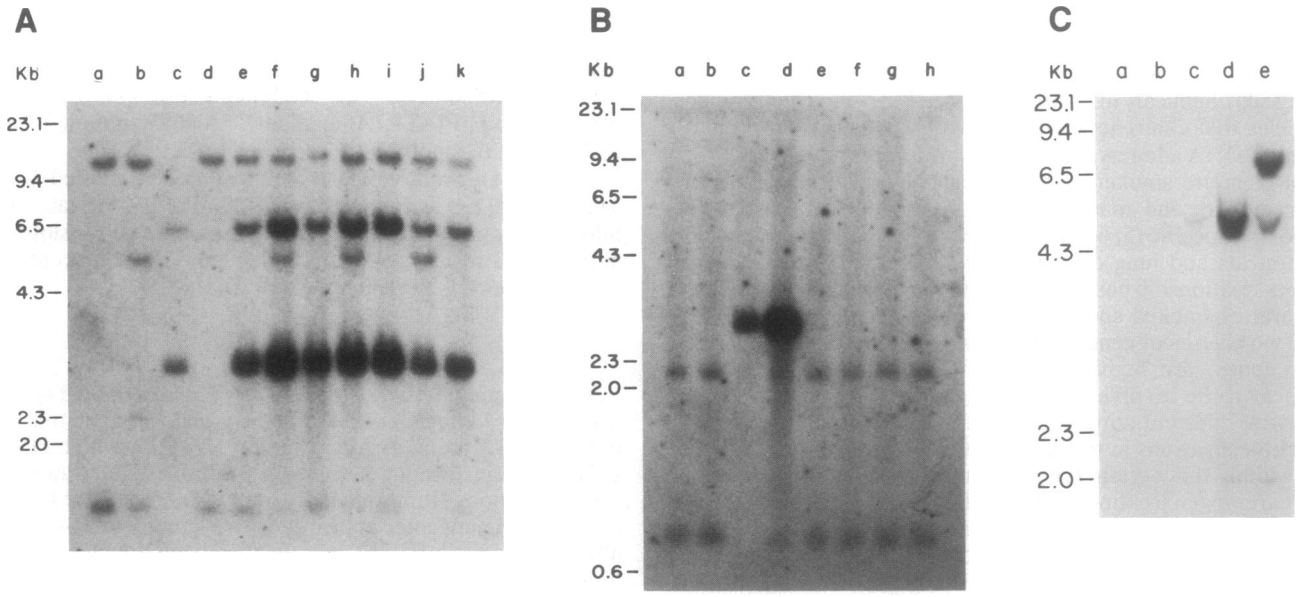

FIG. 1. Southern blot analysis of *K-*, *H-*, and *NRAS* sequences in NIH 3T3 foci and nude mouse tumor DNAs derived from transfection of human lung tumor DNAs. (*A*) Lanes: a, NIH 3T3 DNA; b, nude mouse liver DNA; c, human T24 cell line DNA; d, f, h, and j, primary nude mouse tumor DNAs from lung tumors 3, 31, 36, and 32, respectively; e, i, and k, focus DNAs from lung tumors 31, 32, and 1, respectively; g, cell line DNA from a primary nude mouse tumor DNA from lung tumor 36. The probe was the Kirsten murine sarcoma virus *ras* gene. DNAs were digested with *Eco*RI. (*B*) Lanes: a, NIH 3T3 DNA; b, nude mouse liver DNA; c, human T24 cell line DNA; d, e, g, and h, primary nude mouse tumor DNAs from lung tumors 3, 31, 36, and 32, respectively; f, cell line DNA from a primary nude mouse tumor DNA from lung tumor 36. The probes were the Harvey murine sarcoma virus *ras* gene and the human *HRAS1* gene. The DNAs were digested with *Sac* I. (*C*) Lanes: a, NIH 3T3 DNA; b, nude mouse liver DNA; c, human T24 cell line DNA; d and e, primary nude mouse tumor DNAs from lung tumors 46 and 81, respectively. The probe was the first coding exon of the human *NRAS* gene. DNAs were digested with *Taq* I.

The primary nude mouse tumor DNAs that were *Alu* positive but did not hybridize to the previously mentioned DNA probes were subjected to a second round of DNA transfection in the nude mouse tumorigenicity assay. Five primary nude mouse tumor DNAs from adenocarcinoma 33, two primary nude mouse tumor DNAs from squamous cell carcinoma 40, and one primary nude mouse tumor DNA from adenocarcinoma 41 yielded secondary nude mouse tumors with a very high frequency and short latency relative to the normal control DNAs. All of the secondary nude mouse tumor DNAs were found to contain human *Alu* sequences. Selected secondary nude mouse tumor DNAs were passaged in the nude mouse tumorigenicity assay and also resulted in high tumor incidences and short latency (data not shown).

The third cycle nude mouse tumor DNAs were then analyzed for common *Alu*-containing bands by Southern blot analysis. After *Eco*RI and *Bam*HI double digestion, two highly conserved *Alu*-containing bands are observed at 15.1 kilobases (kb) and 4 kb in DNAs induced by transfection of adenocarcinoma 33 DNA (Fig. 3, lanes a–e). Four highly conserved *Alu*-containing bands are observed at 5.6, 3, 2.6, and 2.4 kb in DNAs induced by squamous cell carcinoma 40 and adenocarcinoma 41 DNAs (lanes f–h). These data suggest that an unidentified transforming gene was repeatedly detected in adenocarcinoma 33 DNA and a second unidentified transforming gene was detected twice in squamous cell carcinoma 40 DNA and once in adenocarcinoma 41 DNA.

Mechanisms of Activation of *RAS* and *RAF* Oncogenes. PCR gene amplification followed by direct dideoxynucleotide DNA sequencing revealed all of the *KRAS* oncogenes in the foci or nude mouse tumor DNAs to be activated by a G → T transversion at the first base of codon 12 (Fig. 4*A*; Table 2). The same activating G → T transversions were detected in the original lung tumor DNAs (Fig. 4*B*). The *NRAS* genes detected in adenocarcinoma 46 and squamous cell carcinoma 81 were found to be activated by an A → T transversion at the second base of codon 61 or a G → A transition at the second base of codon 12 (Table 2), respectively.

The *HRAS* oncogene detected in epidermoid carcinoma 3 was found to contain only the normal DNA sequences in all four coding exons (data not shown). However, the nude mouse tumor DNA containing the lung tumor *HRAS* oncogene was highly efficient at inducing morphological transformation of NIH 3T3 cells in a number of DNA transfection experiments. Southern blot analysis of the human *HRAS* genes detected in these foci DNAs revealed a normal restriction pattern, indicating that there was no truncation of the −1 exon (25). Furthermore, PCR amplification and direct dideoxynucleotide DNA sequence analysis of the splice sites for the IDX intron (26) in the transfected *HRAS* oncogenes showed only the normal DNA sequence at those sites (data not shown).

These foci cell lines produce p21 proteins with normal electrophoretic mobilities when analyzed by SDS/PAGE (27), further suggesting that there are no altered or mutated

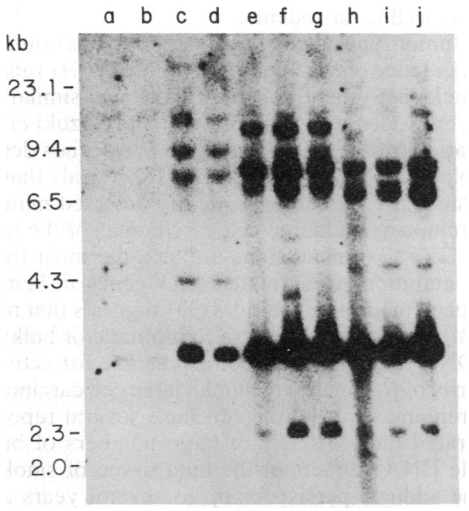

FIG. 2. Southern blot analysis of *raf* sequences in *Eco*RI-digested primary and secondary nude mouse tumor DNAs. Lanes: a, NIH 3T3 DNA; b, nude mouse liver DNA; c, human lung tumor 33 DNA; d, human lung tumor 14 DNA; e, primary nude mouse tumor DNA from lung 14; f and g, secondary nude mouse tumor DNAs derived from primary nude mouse tumor DNA in lane e; h, primary nude mouse tumor DNA from lung 14; i and j, secondary nude mouse tumor DNAs derived from primary nude mouse tumor DNA in lane h. The probe was a human *RAF* cDNA containing all coding exons.

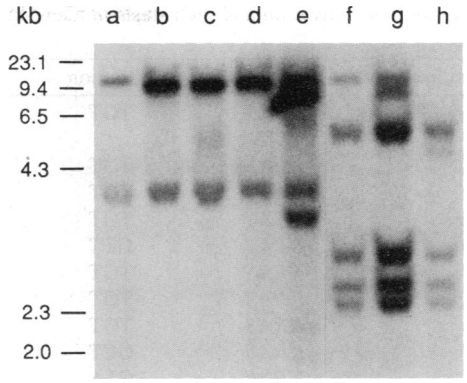

FIG. 3. Southern blot analysis of conserved DNA fragments containing human *Alu* sequences in third cycle nude mouse tumor DNAs that contain unidentified transforming genes. Lanes: a–e, DNAs derived from transfection of lung tumor 33 DNA; f and g, DNAs derived from transfection of lung tumor 40 DNA; h, DNA derived from transfection of lung tumor 41 DNA. DNAs were digested with *Eco*RI and *Bam*HI. The probe was human *Alu*.

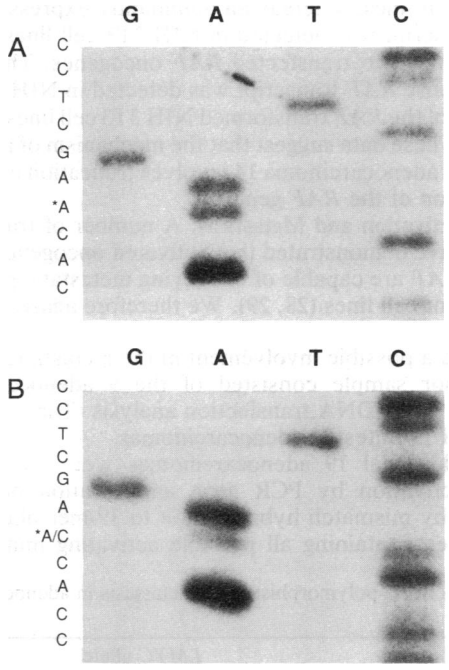

FIG. 4. Identification of a codon 12 *KRAS* mutation by direct sequencing of PCR-amplified DNA. (*A*) Primary nude mouse tumor DNA derived from transfection of lung tumor 32 DNA. (*B*) Human lung tumor 32 DNA. Antisense strand sequences are shown on the left and mutations are noted by nucleotides marked with an asterisk. Note that both the mutated and normal allele are observed in the original lung tumor DNA.

Table 2. Oncogene activation and metastasis in adenocarcinoma of the lung

Adenocarcinoma	Oncogene*	Mutation	TNM†
1	*KRAS*	TGT	$T_3N_0M_0$
14	*RAF*		$T_3N_1M_0$
18	*KRAS*	TGT	$T_2N_1M_0$
31	*KRAS*	TGT	$T_2N_2M_1$
32-B‡	*KRAS*	TGT	$T_3N_0M_0$
32-D‡	*KRAS*	GTT	$T_2N_1M_0$
35	*KRAS*	TGT	$T_1N_0M_0$
36	*KRAS*	TGT	$T_4N_2M_0$
43	*KRAS*	TGT	$T_4N_2M_1$
44	*KRAS*	GCT	$T_3N_2M_1$
46	*NRAS*	CTA	$T_3N_0M_1$
64	*KRAS*	TGT	$T_1N_0M_0$
72	*KRAS*	TGT	$T_2N_0M_0$
95	*KRAS*	GCT	$T_2N_1M_0$

*Data obtained from nude mouse tumorigenicity and focus assays (Table 1) and from analysis of an additional 19 adenocarcinomas analyzed for *KRAS* mutations as described in text.
†TNM, tumor size, regional lymph node metastasis, and metastasis to other organs, respectively.
‡Tumor samples from the Beth Israel Hospital (B) and Duke University Medical Center (D).

forms of *HRAS* protein produced by the transfected *RAS* oncogenes (data not shown).

RAF oncogenes detected in transfection assays have frequently been found to be activated by truncation of the 5′ portion of the gene and subsequent fusion of the remaining 3′ portion of the gene to other cellular sequences. These truncations and fusions result in the expression of aberrant-sized mRNA transcripts for the activated *RAF* genes (22). We therefore analyzed *RAF* mRNA expression in NIH 3T3 cell lines containing the *RAF* oncogenes detected in adenocarcinoma 14 DNA. A normal 3.5-kb *RAF* transcript was detected in the human T24 bladder carcinoma and CaLu-1 lung carcinoma cell lines, whereas an abundantly expressed 3.1-kb *RAF* transcript was detected in NIH 3T3 cell lines carrying either of the two transfected *RAF* oncogenes. The normal 3.3-kb mouse *RAF* transcript was detected in NIH 3T3 cells and in all of the *RAF* transformed NIH 3T3 cell lines (data not shown). These data suggest that the mechanism of activation of *RAF* in adenocarcinoma 14 involves truncation or deletion of a portion of the *RAF* gene.

RAS Activation and Metastasis. A number of transfection studies have demonstrated that activated oncogenes such as *RAS* or *RAF* are capable of conveying metastatic properties to recipient cell lines (28, 29). We therefore analyzed a total of 28 lung adenocarcinomas for *KRAS* gene activation to determine a possible involvement in the metastatic process. This tumor sample consisted of the 9 adenocarcinomas analyzed in the DNA transfection analysis (Table 1) and an additional 19 untested adenocarcinomas.

The additional 19 adenocarcinomas were analyzed for *KRAS* activation by PCR gene amplification of exon 1 followed by mismatch hybridization to 19-mer oligonucleotide probes containing all possible activating mutations at codon 12. A combination of the data from the oligonucleotide mismatch hybridization analysis and the transfection analysis indicated that 13 of 28 (46%) of the adenocarcinomas contained activated *RAS* genes (Table 2). Ten of 28 of the adenocarcinomas had metastasized to regional lymph nodes or other organs at the time of surgery. Eight of 10 of the metastatic adenocarcinomas were found to contain an activated *RAS* gene and 1 of the 2 remaining metastatic adenocarcinomas contained an activated *RAF* gene (Table 2).

Table 3. *LMYC* polymorphism and metastasis in adenocarcinoma of the lung

	LMYC allele		
	10 kb/10 kb	10 kb/6 kb	6 kb/6 kb
Total cases	8	11	9
N(+)	2	4	3
M(+)	0	3	

N(+), regional lymph node metastasis at time of surgery. M(+), metastasis to other organs at time of surgery.

***LMYC* Polymorphism and Metastasis.** Kawashima *et al.* (30) have reported a striking correlation between the presence of a 6-kb *Eco*RI allele of the *LMYC* gene and the speed and extent of metastasis at the time of surgery for Japanese lung cancer patients.

The 28 adenocarcinoma DNAs that were tested for *RAS* gene activation were analyzed by *Eco*RI digestion and Southern blot analysis to determine the distribution of 10- and 6-kb *LMYC* alleles in those tumors (Table 3). We observed a 1.4-fold increase in regional lymph node metastasis in the adenocarcinomas that contained one or two copies of the 6-kb allele of *LMYC* relative to adenocarcinomas that contained only the 10-kb allele of *LMYC* (Table 3).

DISCUSSION

In this report we examined a series of primary human lung tumors from smokers for the presence of oncogenes by two DNA transfection techniques. The NIH 3T3 focus assay detected oncogenes in 3 of 11 tumors analyzed, whereas the NIH 3T3 cotransfection–nude mouse tumorigenicity assay detected oncogenes in 11 of 13 tumors analyzed. Overall, 12 of 14 (86%) of the primary human lung tumors from smokers were found to contain oncogenes (Table 1). With the exception of pancreatic tumors (31, 32), this represents the highest incidence of protooncogene activation reported for tumors of human origin. It is notable that pancreatic and lung cancer, the two human tumor types that exhibit the highest incidences of oncogene activation, have tobacco smoking as a common risk factor (1). The high incidences of oncogene activation in these two tumor types are comparable to those observed in rodent model carcinogenesis systems (10–13) and suggest that protooncogenes are targets for carcinogens in humans as well as in rodents.

Of the tumor types tested, adenocarcinomas contained the highest incidence of *RAS* gene activation (46%) (Tables 1 and 2), an incidence slightly higher than but similar to that reported by Rodenhuis *et al.* (14, 15) and Suzuki *et al.* (16). The mutation profile of the activated *RAS* genes detected in this (Table 2) and other studies (14–16) reveals that G → T transversions are the most frequently detected mutations in adenocarcinomas and large cell carcinomas of the lung. The fact that G → T transversions are also the most frequently detected mutations in activated *RAS* genes in benzo[*a*]pyrene-induced mouse lung tumors (33) suggests that mutagens in cigarette smoke that give rise to aromatic or bulky hydrophobic DNA adducts might be responsible for activation of the majority of *RAS* genes in human large cell carcinomas and adenocarcinomas of the lung. In fact, several reports have demonstrated the existence of large numbers of bulky hydrophobic DNA adducts in the lung tissue of smokers and that these adducts persist for up to several years after the cessation of smoking (5, 6).

Activated *RAS* genes were also detected in other human lung tumor types, notably epidermoid and squamous cell carcinoma (Table 1). These results are in agreement with those reported by Suzuki *et al.*, in which a low level (2 of 13) of squamous cell carcinomas of the lung were found to contain activated *HRAS* genes (16). The absence of detectable alterations in the transfected *HRAS* oncogene from epidermoid carcinoma 3 suggests overexpression of the nor-

mal *RAS* p21 protein as a mechanism of activation. The possibility of *HRAS* gene activation by overexpression in squamous cell tumors is strengthened by the report of Kurzrock *et al.* (34), who have reported that 9 of 11 squamous cell tumors of the lung overexpress *RAS* p21 protein by 4- to 10-fold relative to adjacent normal tissue, whereas only 1 of 9 lung adenocarcinomas overexpressed *RAS* p21 protein. The 5′ flanking region of the human *HRAS* gene contains a number of Sp1 transcription factor binding sites (35) as well as two sets of positive and negative elements that affect the efficiency of *HRAS* oncogene transformation (36). There are seven GC runs within this 5′ flanking region, several of which are within the Sp1 transcription factor binding sites. These GC runs have been shown to be preferred sites of alkylation *in vitro* by guanine alkylating agents (37). This suggests that protooncogenes such as *RAS* and *MYC*, which have extensive GC runs in their promoter regions (37), could be activated by mutations that would affect expression of the normal gene product as well as by mutations that would induce alterations in the coding sequence of the gene. Further molecular analysis of the activated *HRAS* oncogene will be necessary to determine its actual mechanism of activation.

Activated *RAF* genes have been reproducibly detected only in radioresistant, noncancerous skin fibroblasts from a cancer-prone family (38) and in a radiation-resistant human laryngeal cancer cell line (39), indicating that *RAF* is activated at a low but detectable incidence in human tissues. The frequency with which activated *RAF* was detected, 1 of 14 lung tumors (7%), would imply that *RAF* gene activation is a rare event in lung tumors as well as in other human tumor types.

Three of 14 tumors were found to contain unidentified transforming genes (Table 1; Fig. 3). One the basis of the limited sample size, our data indicate that ≈20% of human lung tumors contain transforming genes unrelated to *H-*, *K-*, or *NRAS, RAF, MET, MYC, NEU, ABL*, or the epidermal growth factor receptor. The mechanism and extent of activation of these genes can be more accurately assessed after molecular cloning and identification of the transforming genes.

The metastatic spread of cancer cells from a primary tumor to other sites in the host is a major cause of death in cancer patients. Our finding that 8 of 10 metastatic adenocarcinomas contained an activated *RAS* gene indicates that *RAS* gene activation may be tightly linked to the process of metastasis (Table 2). However, the fact that an additional five adenocarcinomas contained activated *RAS* but were not metastatic suggests that activated *RAS* is not in itself sufficient for the induction of metastasis (Table 2).

The 1.4-fold increase we observed in regional lymph node metastasis in adenocarcinomas that contained one or more copies of the 6-kb allele of *LMYC* (Table 3) was significantly smaller than the 3.4-fold increase reported by Kawashima *et al.* (30). Our data most closely resemble that of Tefre *et al.* (40), who found no significant association between the presence of the 6-kb *Eco*RI allele of *LMYC* and increased risk of metastasis in Norwegian lung cancer patients. A number of the patients included in our study had advanced disease at the time of surgery. Therefore, the discrepancy between our results and those of Kawashima *et al.* (30) could be explained in part if the *LMYC* polymorphism is related to the speed rather than the extent of metastasis.

The high incidence of activated oncogenes in lung tumors (86%) from smokers indicates that oncogene activation is an integral step in the development of certain types of human tumors, especially in those types related to chemical exposure. While it was not possible to determine the stage of tumor development at which protooncogene activation occurred in these human lung tumors, there did appear to be a good correlation between the presence of *RAS* oncogenes and metastatic progression in the lung adenocarcinomas. The similarity and incidence of oncogene activation in our study and in chemically induced rodent lung carcinogenesis studies (33) suggest that rodent models for carcinogenesis have applicability to human tumorigenesis.

1. U.S. Department of Health and Human Services (1982) *The Health Consequences of Smoking: Cancer, A Report of the Surgeon General* (Office on Smoking and Health, Rockville, MD).
2. Fielding, J. (1985) *N. Engl. J. Med.* **313,** 491–498.
3. Stedman, R. L. (1968) *Chem. Rev.* **68,** 153–207.
4. Dube, M. & Green, C. (1982) *Recent Adv. Tobacco Sci.* **8,** 42–102.
5. Phillips, D., Hewer, A., Martin, C., Garner, R. & King, M. (1988) *Nature (London)* **336,** 790–792.
6. Randerath, E., Miller, R., Mittal, D., Avitts, T., Dunsford, H. & Randerath, K. (1989) *J. Natl. Cancer Inst.* **81,** 341–347.
7. Doll, R. & Hill, A. (1952) *Br. Med. J.* **ii,** 1271–1286.
8. Bishop, M. J. (1983) *Annu. Rev. Biochem.* **52,** 301–354.
9. Weinberg, R. (1989) *Cancer Res.* **49,** 3713–3721.
10. Balmain, A., Ramsden, M., Bowden, G. & Smith, J. (1984) *Nature (London)* **307,** 658–660.
11. Guerrero, I., Villasante, A., Corces, V. & Pellicer, A. (1984) *Science* **225,** 1159–1162.
12. Zarbl, H., Sukumar, S., Arthur, A., Martin-Zanca, D. & Barbacid, M. (1985) *Nature (London)* **315,** 382–385.
13. Wiseman, R., Stowers, S., Miller, E., Anderson, M. & Miller, J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5825–5829.
14. Rodenhuis, S., van de Wetering, M., Mooi, W., Evers, S., van Zandwijk, N. & Bos, J. (1987) *N. Engl. J. Med.* **317,** 929–935.
15. Rodenhuis, S., Slebos, R., Boot, A., Evers, S., Mooi, W., Wagennar, S., van Bodegom, P. & Bos, J. (1988) *Cancer Res.* **48,** 5738–5741.
16. Suzuki, Y., Orita, M., Shiraishi, M., Hayashi, K. & Sekiya, T. (1990) *Oncogene* **5,** 1037–1043.
17. Reynolds, S., Stowers, S., Maronpot, R., Anderson, M. & Aaronson, S. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 33–37.
18. Fasano, O., Birnbaum, D., Edlund, L., Fogh, J. & Wigler, M. (1984) *Mol. Cell. Biol.* **4,** 1695–1705.
19. Saiki, R., Gefland, D., Stoffel, S., Scharf, S., Higuchi, R., Horn, G., Mullis, K. & Erlich, H. (1988) *Science* **235,** 487–491.
20. Tindall, K. & Stankowski, L. (1989) *Mutat. Res.* **220,** 241–253.
21. Verlaan-de Vries, M., Bogaard, M., van den Elst, H., van Boom, J., van der Eb, A. & Bos, J. (1986) *Gene* **50,** 313–320.
22. Stanton, V. & Cooper, G. (1987) *Mol. Cell. Biol.* **7,** 1171–1179.
23. Ishikawa, F., Takaku, F., Nagao, M. & Sugimura, T. (1987) *Mol. Cell. Biol.* **7,** 1226–1232.
24. Fukui, M., Yamamoto, T., Kawai, S., Mitsunobu, F. & Toyoshima, K. (1987) *Mol. Cell. Biol.* **7,** 1776–1781.
25. Chichutek, K. & Duesberg, P. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2340–2344.
26. Cohen, J., Broz, S. & Levinson, A. (1989) *Cell* **58,** 461–472.
27. Srivastava, S., Yuasa, Y., Reynolds, S. & Aaronson, S. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 38–42.
28. Liotta, L. (1988) *J. Natl. Cancer Inst.* **80,** 468–469.
29. Egan, S., Wright, J., Jarolim, L., Yanagihara, K., Bassin, R. & Greenberg, A. (1987) *Science* **238,** 202–205.
30. Kawashima, K., Shikama, H., Imoto, K., Izawa, M., Naruke, T., Okabayashi, K. & Nishimura, S. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2353–2356.
31. Almoguera, C., Shibata, D., Forrester, K., Martin, J., Arnheim, N. & Perucho, M. (1988) *Cell* **53,** 549–554.
32. Smit, V., Boot, A., Smits, A., Fleuren, G., Cornelisse, C. & Bos, J. (1988) *Nucleic Acids Res.* **16,** 7773–7782.
33. You, M., Candrian, U., Maronpot, R., Stoner, G. & Anderson, M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 3070–3074.
34. Kurzrock, R., Gallick, G. & Gutterman, J. (1986) *Cancer Res.* **46,** 1530–1534.
35. Ishii, S., Kadonaga, J., Tjian, R., Brady, J., Merlino, G. & Pastan, I. (1986) *Science* **232,** 1410–1413.
36. Honkawa, H., Masahashi, W., Hashimoto, S. & Hashimoto-Gotoh, T. (1987) *Mol. Cell. Biol.* **7,** 2933–2940.
37. Mattes, W., Hartley, J., Kohn, K. & Matheson, D. (1988) *Carcinogenesis* **9,** 2065–2072.
38. Chang, E., Pirollo, K., Zou, Z., Cheung, H., Lawler, E., Garner, R., White, E., Bernstein, W., Fraumeni, J. & Blattner, W. (1987) *Science* **237,** 1036–1039.
39. Kasid, U., Pfeifer, A., Weichselbaum, R., Dritschilo, A. & Mark, G. (1987) *Science* **237,** 1039–1041.
40. Tefre, T., Borresen, A., Aamdal, S. & Brogger, A. (1990) *Br. J. Cancer* **61,** 809–812.