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Activation of Proto-Oncogenes in Human and Mouse Lung Tumors

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Lung cancer is a leading cause of cancer-related deaths in several nations. Epidemiological studies have indicated that 85% of all lung cancer deaths and 30% of all cancer deaths in the U.S. are associated with tobacco smoking. Various chemicals in tobacco smoke are thought to react with DNA and to ultimately yield heritable mutations. In an effort to understand the molecular mechanisms involved in lung tumorigenesis, we have analyzed proto-oncogene activation in a series of human lung tumors from smokers and spontaneously occurring and chemically induced lung tumors in mice. Approximately 86% of the human lung tumors and >90% of the mouse lung tumors were found to contain activated oncogenes. *ras* Oncogenes activated by point mutations were detected in many of the human lung adenocarcinomas and virtually all of the mouse lung adenomas and adenocarcinomas. The mutation profiles of the activated K-*ras* genes detected in the chemically induced mouse lung tumors suggest that the observed mutations result from genotoxic effects of the chemicals. Comparison of the K-*ras* mutations observed in the human lung adenocarcinomas with mutation profiles observed in the mouse lung tumors suggest that bulky hydrophobic DNA adducts may be responsible for the majority of the mutations observed in the activated human K-*ras* genes. Other data indicate that approximately 20% of human lung tumors contain potentially novel transforming genes that may also be targets for mutagens in cigarette smoke.

Introduction

There are four major histologic types of lung cancer: adenocarcinoma (representing 30%), squamous or epidermoid carcinoma (representing 25%), large cell carcinoma (15%), and small cell carcinoma (25%), with uncommon types (such as bronchiolalveolar carcinoma and bronchial carcinoids) and combined types making up the remaining 5% (*1*). Adenocarcinoma, squamous carcinoma, and large cell carcinoma are collectively referred to as "nonsmall cell lung cancer."

Epidemiological studies have indicated that approximately 85% of all lung cancer deaths in the U.S. are associated with tobacco smoking (*2,3*). Tobacco smoke contains more than 3800 chemicals (*2,4*), and approximately 50 of these chemicals have been shown to be mutagenic (*5,6*) or carcinogenic (*2*). It is generally thought that an initial step in lung tumorigenesis in smokers involves the conversion of these chemicals to electrophilic species that can react covalently with cellular macromolecules such as DNA to yield promutagenic DNA adducts. Using ^{32}P-postlabeling techniques, it has been shown that cigarette smokers have higher DNA adduct levels than nonsmokers, and many of the DNA adducts appear to be smoking specific (*7,8*). 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 (*9*).

The process of cell transformation is a multistep phenomenon. Increasing evidence suggests that a small set of cellular genes appear to be targets for genetic alterations that contribute to the neoplastic transformation of cells. The development of neoplasia may, in many cases, require changes in at least two classes of cellular genes: proto-oncogenes (*10*) and tumor-suppressor genes (*11*). For example, both the activation of *ras* oncogenes and the inactivation of several tumor-suppressor genes have been observed in the development of human colon tumors (*12*) and human lung tumors (*13–15*). The activation of proto-oncogenes in chemically induced rodent tumors has been studied in great detail. Investigations in rodent models for chemical carcinogenesis imply that certain types of oncogenes are activated by carcinogen treatment and that this activation process is an early event in tumor induction (*16–18*).

Several studies have detected activated oncogenes in chemically induced rodent lung tumors (*17–20*). Lung tumors from smokers provide a unique opportunity to investigate proto-oncogene activation in a chemical-associated human tumor type. The focus of this paper is to compare and discuss the activation of proto-oncogenes in human and rodent lung tumors with special emphasis on *ras* oncogenes.

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Table 1. Transforming genes in human lung tumors.

DNA source	Focus assay	Tumorigenicity assay[a]	Transforming gene
T24 cell line[b]	+	8/8	H-*ras*
NIH 3T3 cells[c] or normal human lung	−	1/8	
Large cell carcinoma 31	+	8/8	K-*ras*
Adenocarcinoma 1	+	NT	K-*ras*
Adenocarcinoma 14	−	5/16	c-*raf*
Adenocarcinoma 27	−	3/8	
Adenocarcinoma 32	+	11/12	K-*ras*
Adenocarcinoma 33	−	8/20	non-*ras*
Adenocarcinoma 36	−	5/8	K-*ras*
Adenocarcinoma 41	−	5/8	non-*ras*
Adenocarcinoma 43	NT[d]	4/8	K-*ras*
Adenocarcinoma 46	NT	6/8	N-*ras*
Epidermoid carcinoma 3	−	1/8	H-*ras*
Squamous cell carcinoma 11	−	0/8	
Squamous cell carcinoma 40	−	3/8	non-*ras*
Squamous cell carcinoma 81	NT	7/8	N-*ras*

[a]Tumors/injection sites.
[b]Positive control DNA.
[c]Negative control DNA.
[d]NT, not tested.

Results

Detection of Transforming Genes in Human Lung Tumors

We used two general oncogene detection techniques, the NIH 3T3 focus assay and the NIH 3T3 cotransfection nude mouse tumorigenicity assay, to analyze proto-oncogene activation in a series of primary human lung tumors from smokers. Overall, 12 of 14 (86%) of the primary human lung tumors from smokers scored as positive in either the NIH 3T3 focus assay or the NIH 3T3 cotransfection nude mouse tumorigenicity assay (Table 1). The sensitivity of the tumorigenicity assay appeared to be much greater than that of the focus assay for the detection of transforming genes in human lung tumors (Table 1).

Southern blot analysis revealed that five activated K-*ras* genes, one activated H-*ras* gene, and two activated N-*ras* genes were present in eight of the foci or nude mouse tumor DNAs analyzed. Activated K-*ras* genes were detected in one large cell carcinoma and four adenocarcinomas, activated H-*ras* was detected in one epidermoid carcinoma, and activated N-*ras* was detected in an adenocarcinoma and a squamous cell carcinoma (Table 1). An activated *raf* gene was detected in one adenocarcinoma and transforming genes that did not hybridize to probes specific for H-, K-, or N-*ras*, c-*raf*, *met*, *neu*, c-*myc*, c-*abl*, or the epidermal growth factor (EGF) receptor were detected in two adenocarcinomas and one squamous cell carcinoma (Table 1).

Table 2. *ras* Gene mutations detected in human lung tumors.

Tumor type	Number[a]	Oncogene	Activating mutation
Large cell carcinoma	1	K-*ras*	GGT → TGT
Adenocarcinoma	9	K-*ras*	GGT → TGT
Adenocarcinoma	2	K-*ras*	GGT → GCT
Adenocarcinoma	1	K-*ras*	GGT → GTT
Adenocarcinoma	1	N-*ras*	CAA → CTA
Squamous cell carcinoma	1	N-*ras*	GGT → GAT
Epidermoid carcinoma	1	H-*ras*	?

[a]Total number of tumors containing a particular mutation.

Mechanisms of Activation of *ras* Oncogenes in Human Lung Tumors

ras Genes are usually activated *in vivo* by point mutations at codon 12, 13, 61, 117, or 146 (*21–23*). We therefore used the polymerase chain reaction (PCR) gene amplification technique to amplify specific *ras* exons and performed direct dideoxy DNA sequencing to determine the position of activating mutations in the transfected *ras* oncogenes. All of the K-*ras* oncogenes in the foci or nude mouse tumor DNAs were found to be activated by a G:T transversion at the first base of codon 12 (GGT → TGT) (Table 2). The same activating G:T transversions were detected in the original lung tumor DNAs when analyzed by PCR amplification and direct dideoxy DNA sequencing or oligonucleotide mismatch hybridization. The N-*ras* genes were found to be activated by a A:T transversion at the second base of codon 61 (CAA → CTA) or by a G:A transition at the second base of codon 12 (GGT → GAT) (Table 2). The H-*ras* oncogene detected in epidermoid carcinoma 3 was found to contain only the normal DNA sequences in all four coding exons and therefore appears to be activated by a novel mechanism. An additional 19 adenocarcinomas were analyzed for K-*ras* 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. Eight of 19 adenocarcinomas were found to contain point mutations at codon 12 (5 GGT → TGT, 2 GGT → GCT, 1 GGT → GTT) (Table 2).

Table 3. Mutation spectrum in K-*ras* oncogenes detected in spontaneous and chemically induced mouse lung tumors.[a]

Treatment[b]	No. of tumors with K-*ras*	Codon 12 (GGT) Mutations			Codon 13 (GGC) Mutations		Codon 61 (CAA) Mutations			
		TGT	GTT	GAT	CGT	CGC	CTA	CGA	CAT	UN
Spontaneous	19	0	4	4	1	0	0	7	2	1
BP	14	8	1	4	0	0	0	0	0	1
DMBA	10	0	0	0	0	0	10	0	0	0
EC	10	0	1	0	0	0	7	2	0	0
MNU	15	0	0	15	0	0	0	0	0	0
NMK	11	0	1	8	0	0	0	2	0	0
DMN	10	0	0	7	0	0	0	3	0	0
TNM	4	0	0	4	0	0	0	0	0	0
BD	6	0	0	0	0	6	0	0	0	0

[a]Data from Stowers et al. (*17*), You et al. (*18*), Belinsky et al. (*19*), Goodrow et al. (*20*), and unpublished observations.
[b]Abbreviations: BP, benzo[*a*]pyrene; DMBA, dimethylbenz[*a*]anthracene; EC, ethyl carbamate; MNU, methylnitrosourea; NMK 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone; DMN, dimethylnitrosamine; TNM, tetranitromethane; BD, 1,3-butadiene; UN, unidentified mutation.

ras Gene Activation in Spontaneous and Chemically Induced Mouse Lung Tumors

A number of recent reports from our laboratory have demonstrated K-*ras* gene activation in spontaneous or chemically induced A/J mouse lung tumors (*18,19*) and in chemically induced B6C3F$_1$ mouse lung tumors (*17,20*). The mouse lung tumors from these studies were classified as adenomas or adenocarcinomas and were morphologically similar to lung tumors of the same type in humans (*17–20*). The K-*ras* gene activation data from these studies are summarized in Table 3.

Discussion

Recent studies (*14,24*) have speculated that mutational activation of the K-*ras* oncogene detected in human lung adenocarcinomas might be a direct effect of one or more carcinogenic ingredients of tobacco smoke. Rodenhuis et al. found that 15 of 45 (33%) of the adenocarcinomas from smokers contained activated *ras* genes, whereas 6 of 6 (100%) of the adenocarcinomas from patients who had never smoked did not contain an activated *ras* gene (*14,24*). Thus, carcinogens in tobacco smoke might be a major causative factor in the induction of the *ras* gene point mutations in human lung adenocarcinomas. Furthermore, the reproducible activation of K-*ras* oncogenes in chemically induced lung tumors from different strains of mice has made it possible to correlate the activating mutations with the promutagenic adducts formed directly or by metabolic activation of the carcinogen. The selectivity of mutations in the K-*ras* oncogene observed in chemically induced A/J mouse lung tumors, as compared to spontaneous tumors, suggests that these carcinogens directly induced point mutations in the K-*ras* proto-oncogene (Table 3) (*18,19*).

The mutation profile of the activated *ras* genes detected in this (Table 2) and other studies (*14,15,24*) reveals that G:T transversions are the most frequently detected *ras* gene 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 (*18*) (Table 3) 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 lung tissue of smokers and that these adducts persist for up to several years after the cessation of smoking (*7,8*).

The high incidence of activated oncogenes in human lung tumors from smokers and chemically induced mouse lung tumors (Tables 1 and 3) indicates that oncogene activation is an integral step in the development of certain types of tumors, especially in those types related to chemical exposure. The detection of potentially novel transforming genes in approximately 20% of human lung tumors from smokers (Table 1) suggests that genes other than H-, K-, or N-*ras* are putative subtrates for mutagenic activation by carcinogens in tobacco smoke.

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