Role of Proto-oncogene Activation in Carcinogenesis

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*The accumulation of genetic damage in the forms of activated proto-oncogenes and inactivated tumor-*suppressor genes is the driving force in the evolution of a normal cell to a malignant cell. For example, both the activation of *ras* oncogenes and the inactivation of several suppressor genes, including *p53*, have been observed in the development of human colon and lung tumors. Point mutations in key codons can activate *ras* proto-oncogenes and inactivate the *p53* suppressor gene. Thus, several critical genes for tumorigenesis are potential targets for carcinogens and radiation that can induce point mutations at low doses. The *ras* proto-oncogenes are targets for many genotoxic carcinogens. Activation of the *ras* gene is an early event—probably the "initiating" step—in the development of many chemical-induced rodent tumors. *ras* Oncogenes are observed in more human tumors and at a higher frequency than any other oncogene, and activation of the proto-oncogene may occur at various stages of the carcinogenic process. Numerous proto-oncogenes other than the *ras* genes have been shown to be activated in human tumors and to a lesser extent in rodent tumors. Mechanisms that induce aberrant expression of proto-oncogenes are gene amplification and chromosomal translocation or gene rearrangement. Amplification of proto-oncogenes and possibly gene overexpression during the absence of gene amplification occur in the development of many human tumors. For a specific tumor type, amplification of any one proto-oncogene may occur at a low frequency, but the frequency of tumors in which at least one proto-oncogene is amplified can be much higher. Proto-oncogene amplification is usually associated with late stages of tumor progression; however, amplified HER2/*neu* has been observed in early clinical stages of mammary neoplasia. Activation of proto-oncogenes by chromosomal translocation has been detected at a high frequency in several hematopoietic tumors. Non-*ras* genes have been detected by DNA transfection assays in both human and rodent tumors. For example, *ret* and *trk* genes were found to be activated by gene rearrangements in human papillary thyroid carcinomas. Several potentially new types of oncogenes have also been detected by DNA transfection assays. The etiology of the genetic alterations observed in most human tumors is unclear at present. Examples of *ras* gene activation and those documented for mutations in the *p53* gene demonstrate that exogenous conditions can induce oncogenic mutants of normal genes. The genetic alterations observed in most human tumors are probably generated by both spontaneous events and exogenous conditions.

Introduction

An increasing amount of evidence suggests that a small set of cellular genes appears to be the target 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 gene: proto-oncogenes (*1–12*) and tumor-suppressor genes (*13–16*). For example, activation of the K-*ras* oncogene and inactivation of at least three tumor suppressor genes have been observed in the development of human colon tumors (*17*). This report will discuss the activation of proto-oncogenes in human and rodent tumors and the role of these oncogenes in the etiology and development of tumors.

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Proto-oncogenes

Proto-oncogenes are expressed during "regulated growth," such as embryogenesis, wound healing, regeneration of damaged liver, and stimulation of cell mitosis by growth factors. Proto-oncogenes are highly conserved, being detected in species as divergent as yeast, Drosophila, and humans. These genes encode for growth factors, growth factor receptors with tyrosine kinase activity, regulatory proteins in signal transduction, nonreceptor tyrosine kinases, serine/threonine kinases, and transcription factors (Table 1). The encoded proteins play a crucial role in cellular growth and differentiation (*1,5–8*) and in apoptosis or programmed cell death (*18*).

Proto-oncogenes were initially identified as the transduced oncogenes of acute transforming retroviruses (*9*).

Table 1. Biological functions of cellular proto-oncogenes.

Function	Proto-oncogenes
Growth factor	*sis* (PDGF), *int-2*, *hst-1*
Growth factor receptor with tyrosine kinase activity	*erb* B (EGF receptor), *fms* (CSF receptor), *met* (HGF receptor), *neu*, *ros*, *trk*, *ret*
Tyrosine kinase	*src*, *abl*, *lck*, *yes*
Regulatory protein in signal transduction	Ha-*ras*, K-*ras*, N-*ras*, *gsp*, *gip*
Serine/threonine kinase	*mos*, *raf*
Nuclear regulatory protein	*myc*, *myb*, *fox*, *c-jun*, *rel*

Viral oncogenes arise by recombination between cellular proto-oncogenes and the genome of nontransforming retroviruses. Proto-oncogenes can also be activated to cancer-causing oncogenes by mechanisms independent of retroviral involvement (*1,3,10–12*). These mechanisms include point mutations and gross DNA rearrangements such as translocation and gene amplification, and it is these mechanisms that generate the oncogenes observed in human and rodent tumors.

The *ras* family of proto-oncogenes is of particular interest since these genes have been implicated in the development of numerous tumors. H-*ras*, K-*ras*, and N-*ras* can acquire transforming activity by a point mutation in their coding sequence. *In vivo*, activating point mutations have been observed in codons 12, 13, 61, 117, and 146 (*19–21*). We have recently detected a 30-base repeat around codon 61 in the K-*ras* gene in several mouse tumors; this repeat appears to activate the K-*ras* gene.

The *ras* gene products are 21,000-Da proteins (p21) which bind guanine nucleotides with high affinity and are thought to be involved in various signal transduction pathways in many cell types (*22–24*). The p21:GDP complex receives a signal from an upstream element (i.e., an activated membrane-bound receptor), and the GDP is exchanged for GTP to convert the inactive p21:GDP complex to the active p21:GTP complex (*25*). The p21:GTP can transmit the signal downstream to an appropriate target. The active GTP complex with p21 is converted to the inactive GDP complex by hydrolysis of GTP to GDP. The p21 protein itself possesses intrinsic GTPase activity; however, *in vivo*, this intrinsic activity is very slow unless enhanced by GTPase-activating protein (GAP). GAP can enhance the GTPase activity of p21 by at least a factor of 4×10^3. The first observed difference in biological activity between a mutant oncogenic p21 and wild-type p21 was the intrinsic GTPase activity, which was 10 times higher in wild-type than in the valine-12 oncogenic mutant. It soon became clear, however, that intrinsic GTPase activity could not be used to differentiate between some mutant forms of p21 observed in human tumors and the wild-type *ras* protein (*26*). After the discovery of GAP, it was shown that the main biochemical difference between oncogenic p21s with mutations in codon 12, 13, or 61 and wild-type p21 is the ability of GAP to induce GTP hydrolysis in the active p21:GTP complex. The GAP-induced hydrolysis can be as much as 1000 times greater in the wild-type p21 than in these mutant forms of *ras* (*27,28*). The mutant forms thus remain in the active GTP form much longer than the wild type, and presumably the continual transmission of a signal by the mutant forms is responsible, at least in part, for the oncogenic properties. The mutations in codon 117 and 146 increase the GDP:GTP exchange rate and thus increase the amount of cellular p21:GTP complex in the absence of an external signal, since the cellular concentration of GTP is much greater than that of GDP. The codon 117 and 146 mutations have been observed only in rodent tumors. The crystal structure of p21 and some of the mutant oncogenic p21s have recently been determined by two groups (*29–32*). Although some structural differences are observed between the oncogenic and wild-type p21, the X-ray crystal structural differences between wild-type p21 and oncogenic mutants do not clearly explain the dramatic differences in response to GAP (*30,31*).

ras Oncogenes in Human and Rodent Tumors

A *ras* oncogene was the first activated proto-oncogene detected in a human tumor, and the K-, H-, and N-*ras* oncogenes have been detected in more human tumor types and at a higher frequency than any other oncogene (Table 2). Activated *ras* proto-oncogenes have been detected in a relatively high percentage of colon carcinomas (47%), pancreatic carcinomas (81%), lung adenocarcinomas (32%), cholangiocarcinomas (88%), certain types of thyroid tumors (56%), endometrical adenocarcinomas (47%), mucinous adenocarcinomas of the ovary (75%), squamous-cell carcinomas (SCC) at sun-exposed body sites (47%), oral SCC associated with tobacco/quid chewing (35%), and to a lesser extent in several other types of human tumor. In some tumor types, however, detection of a *ras* oncogene is a rare event; these include mammary adenocarcinomas (1/55), hepatocellular carcinomas (2/57), cervical carcinomas (1/24), and oral SCC from patients who do not chew tobacco (1/32).

Activated *ras* proto-oncogenes have also been detected in spontaneous and chemical-induced tumors generated in numerous rodent model systems (Table 3) (*3,19,73–79*). The reproducible activation of *ras* oncogenes in chemical-induced rodent tumors has made it possible to correlate the activating mutations with the promutagenic adducts formed either directly or by metabolic activation of the carcinogen. For example, mutational spectra have been determined in *ras* oncogenes detected in tumors generated in mouse lymphocytes (*78*), mouse liver (*20,96*), mouse and rat skin (*76,93*), mouse and rat lung (*81,82,89*), and rat mammary gland (*84,85*). In most of these cases, the observed mutational spectrum in the *ras* oncogenes is consistent with the pattern of DNA adducts induced by the genotoxic carcinogen and is also consistent with *in-vitro* mutation patterns, such as those generated in the *lacI* gene of *Escherichia coli* by methylating agents and benzo[*a*]pyrene (*81*). One striking example is that in which there is selectivity for the *ras* mutation spectrum but no correlation with the known DNA adducts. Activation of H-*ras* and K-*ras* oncogenes in the second base of codon 61, A:T to T:A or G:C, by ethyl carbamate or vinyl carbamate (the putative proximal carcinogenic metabolite of ethyl carbamate) has been observed in mouse skin (*97*), mouse

Table 2. *ras* Oncogenes detected in human tumors.[a]

Tumor	No. positive/ no. tested	Oncogene[b]	References
Colon			
Adenomas (FAP)	10/115	K-*ras* (10)	(*33,34*)
Adenomas	36/84	K-*ras* (35), N-*ras* (1)	(*34–36*)
Carcinomas	136/289	K-*ras* (131), N-*ras* (5)	(*34–38*)
Metastases	35/61	K-*ras* (32), N-*ras* (3)	(*35,39*)
Ulcerative colitis[c]	1/17	K-*ras* (1)	(*38*)
Pancreatic carcinomas	174/220	K-*ras* (174)	(*35,38,40–43*)
Lung adenocarcinomas	65/207	K-*ras* (59), H-*ras* (2), N-*ras* (4)	(*44–47*)
Lung SCC	4/60	H-*ras* (3), N-*ras* (1)	(*44,48*)
Thyroid			
Adenomas[d]	20/29	H-*ras* (14), N-*ras* (5), K-*ras* (1)	(*49,50*)
Follicular carcinomas[d]	11/21	H-*ras* (7), N-*ras* (3), K-*ras* (1)	(*49,50*)
Undifferentiated carcinomas[e]	6/10	H-*ras* (3), N-*ras* (1), K-*ras* (2)	
Adenomas[e]	2/12	H-*ras* (1), N-*ras* (1)	(*49*)
Follicular carcinomas[e]	1/10	H-*ras* (1)	(*49*)
Papillary carcinomas[f]	2/51	N-*ras* (2)	(*49,51,52*)
AML	12/45	K-*ras* -(10), N-*ras* (2)	(*53*)
Pre-AML cells, MDS	3/8	N-*ras* (3)	(*54*)
Pre-AML cells, MDS	2/4	K-*ras* (2)	(*55*)
CML, chronic phase	1/6	H-*ras* (1)	(*56*)
CML, blast phase	3/6	H-*ras* (2), N-*ras* (1)	(*56*)
SCC[g]	15/32	H-*ras* (15), K-*ras* (1)	(*57,58*)
Basal-cell carcinomas[g]	5/16	H-*ras* (5)	(*58*)
Keratoacanthomas	16/50	H-*ras* (16)	(*59*)
Cutaneous melanomas[h]	7/37	N-*ras* (7)	(*60*)
Oral SCC[i]	20/57	H-*ras* (20)	(*61*)
Oral SCC[j]	1/32	H-*ras* (1)	(*62,63*)
Hepatocellular carcinomas	2/57	K-*ras* (1), N-*ras* (1)	(*64–66*)
Cholangiocarcinomas	21/24	K-*ras* (21)	(*66,67*)
Bladder carcinomas	7/62	H-*ras* (7)	(*68,69*)
Endometrial adenocarcinomas	15/42	K-*ras* (14), N-*ras* (1)	(*70,71*)
Cervical carcinomas	1/28	K-*ras*	(*70,71*)
Ovary			
Mucinous adenocarcinomas	9/12	K-*ras* (9)	(*70,71*)
Differentiated nonmucinous	3/28	K-*ras* (3)	(*70,71*)
Invasive mammary adenocarcinomas	1/55	K-*ras* (1)	(*72*, unpublished observations)

Abbreviations: FAP, familial polyposis coli patients; SCC, squamous-cell carcinoma; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia.

[a]The basis for detection of the oncogenes listed in this table is the DNA transfection assay and biochemical assays such as the polymerase chain reaction and the RNAse-A-mismatch-cleavage method.

[b]Numbers in parentheses indicate the number of samples with that oncogene.

[c]Carcinomas and dysplasia arising in patients with ulcerative colitis.

[d]From iodide-deficient areas.

[e]From areas of normal dietary iodide intake.

[f]From both iodide-deficient and normal iodide intake areas.

[g]Sun-exposed body sites.

[h]Tumors positive for *ras* activation were localized on body sites continuously exposed to sunlight.

[i]From Indian patients who chewed tobacco/quid.

[j]From patients in the United Kingdom who did not chew tobacco, although some were smokers.

liver (*96*), and mouse lung (*81*) tumors; however, the only reported adduct to date is a deoxyguanosine adduct (*98–100*). Detailed discussions of the relationship between *ras* mutational spectra observed in chemical-induced tumors, and the known DNA binding properties of the chemical are presented in some of the above-mentioned references (*20,76,78,81,84,85,93,96*).

Many chemicals that induce tumors in rodents have no detectable DNA damaging activity in genotoxicity assays *in vitro* (*101*). Classical "nongenotoxic" tumor promoters such as 12-*O*-tetradecanoylphorbol-13-acetate, phenobarbital, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) can confer a selective growth advantage to cells initiated by genotoxic agents; however, nongenotoxic chemicals, including some of the classical tumor promoters, can induce tumors in rodent model systems without the help of exogenous genotoxic chemicals. The mouse liver is a model in which many nongenotoxic chemicals induce hepatocellular neoplasia (*101*): chemicals that have this property include TCDD, phenobarbital, polychlorinated biphenyls, furan, tetrachloroethylene, trichloroethylene, the class of chemicals designated as peroxisome proliferators (which include many therapeutic agents and industrial agents), and chlorinated insecticides such as chlordane. Since spontane-

Table 3. *ras* Oncogenes and activating mutations detected in rodent tumors.

Condition	Rodent[a]	Tumor	Oncogene (frequency)	Activation mutations[b]	References
Spontaneous	$B6C3F_1$	Lung	K-*ras* (3/16)	$G^{35} \rightarrow A$	(*80*, unpublished observations
Spontaneous	A/J	Lung	K-*ras* (19/20)	$G^{35} \rightarrow T,A;A^{182} \rightarrow G$	(*81,82*)
Spontaneous	$B6C3F_1$	Liver	H-*ras* (67/103)	$C^{181} \rightarrow A;A^{182} \rightarrow G,T$	(*20,83*, unpublished observations)
Spontaneous	C57	Liver	H-*ras* (2/12)	$A^{182} \rightarrow T$	(unpublished observations)
MNU	Rat[c]	Mammary	H-*ras* (61/70)	$G^{35} \rightarrow A$	(*84,85*)
MNU	Wistar rat	Thyroid	H-*ras* (14/15)	ND	(*86*)
Ionizing radiation	Wistar rat	Thyroid	K-*ras* (9/15)	ND	(*86*)
DMN-OME	Fischer rat	Kidney	K-*ras* (10/35)	$G^{35} \rightarrow A$	(*87*)
MBNA	Fischer rat	Esophagus	H-*ras* (18/18)	$G^{35} \rightarrow A$	(*88*)
Tetranitromethane	$B6C3F_1$	Lung	K-*ras* (10/10)	$G^{35} \rightarrow A$	(*89*)
Tetranitromethane	Fischer rat	Lung	K-*ras* (18/19)	$G^{35} \rightarrow A$	(*89*)
1,3-Butadiene	$B6C3F_1$	Lung	K-*ras* (6/9)	$G^{37} \rightarrow C$	(*90*)
BP	A/J	Lung	K-*ras* (14/16)	$G^{34} \rightarrow T$ $G^{35} \rightarrow A$	(*81*)
BP	NIH/Swiss	Skin	H-*ras* (2/5)	$G^{35} \rightarrow T$	(*73*)
DMBA	NIH/Swiss	Skin	H-*ras* (58/61)	$A^{182} \rightarrow T$	(*76*)
DMBA	Rat C	Mammary	H-*ras* (6/29)	$A^{182} \rightarrow N$	(*85*)
DMBA	Rabbit	Skin	H-*ras* (5/10)	$A^{162} \rightarrow T$	(*91*)
DMBA	Rabbit	Skin[d]	H-*ras* (10/17)	$A^{162} \rightarrow T$	(*59,91*)
BOP	Hamster	Pancreas	K-*ras* (10/10)	$G^{35} \rightarrow A$	(*92*)
Benzidine congeners and dyes	Fischer rat	Skin and other	H-*ras* (31/50) N-*ras* (3/50)	$G^{37} \rightarrow C;C^{181} \rightarrow A$	(*93*)
Benzidine	$B6C3F_1$	Liver	H-*ras* (13/22)	$C^{181} \rightarrow A$	(*83*)
Aflatoxin B_1	Fischer rat	Liver	K-*ras* (3/13) N-*ras* (5/13)	$G^{34} \rightarrow T;G^{35} \rightarrow A$	(*94,95*)
Furan	$B6C3F_1$	Liver	H-*ras* (10/29) K-*ras* (2/29)	$C^{161} \rightarrow A;G^{351} \rightarrow C,T$	(*20*)
Tetracholorethylene	$B6C3F_1$	Liver	H-*ras*(11/40) K-*ras* (6/40)	$C^{161} \rightarrow A;A^{162} \rightarrow T$	(unpublished observations)
Phenobarbital	$B6C3F_1$	Liver	H-*ras*(1/15)	$C^{181} \rightarrow A$	(*83*)

Abbreviations: MNU, *N*-methyl-*N*-nitrosourea; DMN-OME methyl(methoxymethyl)nitrosamine; MBNA, methylbenzylnitrosamine; BP, benzo[*a*]pyrene; DMBA, 7,12-dimethylbenz[*a*]anthracene; BOP, *N*-nitrosobis(2-oxopropyl)amine; ND, not determined.

[a]Mouse strains were used unless otherwise noted.

[b]Only mutations that occur at relatively high frequency.

[c]Buf/N, Sprague-Dawley, and Fischer 344 strains.

[d]Keratoacanthomas.

ously occurring tumors in the $B6C3F_1$ mouse strain have a high incidence of activated H-*ras* genes, the pattern of activated *ras* genes in spontaneous tumors and in those induced by nongenotoxic chemicals has been compared in several studies to help determine the mechanisms by which nongenotoxic chemicals induce tumors (*20,83*). For example, trichloroethylene appears to promote the "spontaneously initiated cells" that develop into tumors in untreated animals, whereas furan and tetrachloroethylene induce *ras* mutational spectra distinct from those observed in spontaneous tumors (*20*; unpublished observations). In contrast, phenobarbital appears to select against *ras*-initiated cells in that only 1 of 15 tumors induced by this compound had H-*ras* oncogenes compared to 66% in spontaneous tumors (*83*). Additional studies are required to delineate the mechanisms by which these various nongenotoxic chemicals induce mouse liver tumors as well as tumors in other rodent model systems.

The spectrum of K-*ras* codon 12/13 mutations detected in human lung, colon, and pancreatic tumors is shown in Table 4. Even though the data in Table 4 represent diverse population groups, lung and colon tumors show a distinct difference in their spectra. In lung adenocarcinomas, the G to T mutation in the first base of codon 12 occurs at the highest frequency (53%), whereas this mutation accounts

Table 4. Spectrum of K-*ras* codon 12/13 mutations in human tumors.[a]

Mutation	Colon[b] (n = 166)	Lung (n = 75)	Pancreas (n = 168)
K12 (GGT)			
TGT	10[c]	53	11
GTT	20	15	28
GAT	38	15	39
AGT	7	1	1
CGT	1	4	18
GCT	2	7	1
K13 (GGC)			
GAC	22	3	1
TGC	0	1	0
CGC	0	1	0

[a]Colon tumors consist of adenomas and adenocarcinomas, and lung and pancreatic tumors are adenocarcinomas. Data obtained from references in Table 2 and M. Perucho (personal communication).

[b]Data from Burmer et al. (*38*) are not included, since they observed mainly GGT to AGT mutations in codon 12 (32 of 37 total tumors with *ras* mutations) and only 4 of 37 with G to T mutations. Thus, their mutational spectrum is very different from that observed in the other studies of colon tumors.

[c]Numbers are percentages of total number of K-*ras* 12/13 mutations.

for only 10% of the mutations in colon tumors. In contrast, G to A mutations in the second base of codon 12 or 13 account for 60% of the mutations in colon tumors and for only 18% of the mutations in lung tumors. Pancreatic tumors have similar frequencies of G to T (39%) and G to A (41%) mutations. The K-*ras* oncogene mutational spectra observed in pancreatic carcinomas show considerable variability between individual studies (*38*); the spectra in lung and colon tumors are similar in the various investigations, except for that of Burmer et al. (*38*), noted in Table 4. This does not imply that the data of Burmer et al. are incorrect but that further analysis of their population group would be of interest.

The difference in the K-*ras* mutational spectrum in colon and lung is probably mainly a consequence of cigarette smoking. Although the association of SCC and small-cell carcinomas of the lung (SCLC) with cigarette smoking is well established, only recently has a clear association with adenocarcinomas been suggested (*102*). Philips et al. (*103*) have shown a linear relationship between numbers of cigarettes smoked and DNA adduct levels in the lung. Thus, the activated *ras* genes detected in human pulmonary adenocarcinomas from smokers probably result from a direct genotoxic effect of carcinogens present in tobacco smoke. Comparison of the frequency of detection of *ras* genes in smokers and nonsmokers strongly supports this hypothesis: An activated *ras* proto-oncogene was not detected in six of six nonsmokers or in three of four adenocarcinomas from patients who had stopped smoking at least 5 years before diagnosis, whereas 44% (27/62) of adenocarcinomas from smokers had activated *ras* genes (data from references *44* and *45*, in which the status of smoking was given). The pattern of mutations in K-*ras* oncogenes detected in lung tumors induced in mice by benzo[*a*]pyrene is very similar to that observed in human pulmonary adenocarcinomas (Tables 3 and 4). Colon tumorigenesis in humans is not associated with cigarette smoking. Cigarette smoking represents the most consistently observed risk factor for induction of pancreatic tumors, although the small relative risk associated with cigarette smoke indicates that other conditions are important. The G to T mutations observed in pancreatic tumors could arise, in part, from cigarette smoking.

Several recent studies have examined the patterns of the *p53* gene mutations detected in human tumors (*104,105*). G:C to T:A mutations are the most frequently observed (57%) mutations in the *p53* gene from non-SCLC (*104*), and this pattern is thus similar to that of K-*ras* mutation in pulmonary adenocarcinomas (Table 4). Even though the *p53* mutations were detected mainly in SCC and the K-*ras* mutations mainly in adenocarcinomas, it is very noteworthy that the same mutational pattern is observed in two cancer-causing genes detected in tumors associated with cigarette smoking. Comparison of *p53* mutations detected in colon tumors with the K-*ras* mutations in this tumor type reveals not only a similarity but also a distinct difference. G:C to A:T transitions are the predominant mutations in both genes. This mutation in the *p53* gene occurs at CpG dinucleotides and could result from spontaneous deamination of the 5′-methylcytosine residues (*104*); however, this type of mechanism cannot account for the G:C to A:T transitions observed in the K-*ras* oncogenes detected in colon tumors.

In addition to possible activation of the K-*ras* proto-oncogene by chemicals in cigarette smoke, several other examples are presented in Table 2 in which *ras* genes were probably activated by exposure of humans to exogenous agents: *a) ras* Oncogenes were detected in subcutaneous SCC, basal-cell carcinomas, and melanomas from sun-exposed areas of the body but at a much lower frequency in tumors obtained from unexposed sites; moreover, the mutations observed in H-*ras* oncogenes detected in SCC and basal-cell carcinomas are consistent with ultraviolet radiation-induced DNA lesions (*58*). *b)* Oral SCC obtained from Indian patients who chewed tobacco/quid contained activated H-*ras* genes, whereas oral SCC from patients who did not chew tobacco had a very low incidence of H-*ras* oncogenes. *c)* A high incidence of *ras* oncogenes was observed in thyroid follicular carcinomas and adenomas from people living in iodide-deficient areas, and they were detected at a much lower frequency in these types of tumor obtained from people living in geographical areas where iodide consumption was normal. *ras* Oncogenes were detected at a very low frequency in thyroid papillary carcinomas, without regard to the levels of iodide intake. Thus, iodide deficiency is related to high rates of *ras* activation in thyroid follicular carcinomas and adenomas. These examples of *ras* gene activation and those documented for mutations in the *p53* gene (*104*) demonstrate that exposure of humans to environmental agents or abnormal dietary conditions can induce oncogenic mutants of normal genes.

Several conclusions about the role of *ras* genes in carcinogenesis can be inferred from the data and references presented in Tables 2–4. First, activation of *ras* proto-oncogenes is an early event in many rodent tumors. This deduction is based on several observations: *a) ras* mutations are chemical specific and are observed after a single dose of carcinogens; *b) ras* oncogenes are detected in adenomas, which can give rise to carcinomas; *c) ras* mutations have been detected in preneoplastic lesions in mouse lung and liver (unpublished data), and the same mutations are detected in the carcinomas; *d) ras* mutations have been detected very soon after exposure to a carcinogen—in rat mammary tissue 2 weeks after neonatal exposure to *N*-methyl-*N*-nitrosourea (*106*) and in mouse lung tissue several days after exposure to this nitrosamine (unpublished data). *ras* Activation is probably the initiating event in the development of many chemical-induced tumors; moreover, a cell with an activated *ras* gene can exist in a tissue until endogenous or exogenous factors promote clonal expansion of the initiated cell.

Second, activation of the *ras* gene in human tumors may occur at various stages of the carcinogenic process. *ras* Oncogenes were detected in colon adenomas, and in all but one sample the K-*ras* oncogene detected in the carcinoma was also present in the surrounding adenoma (*37,107*). *ras* Oncogenes have been detected in myelodysplastic syndrome and could be involved in progression to malignant acute myelogenous leukemia (*54,55*). *ras* Oncogenes are

probably a late event in the development of cholangiocarcinoma because they are detected in less than 10% of the cells in the carcinoma (*67*). *ras* Oncogenes are detected in colon metastases (Table 2), and a recent report suggested that *ras* activation may be involved in the metastatic process, as in some cases mutations detected in the metastases were different from those observed in the primary tumor (*39*).

Third, in addition to being observed in malignant tumors and in adenomas, which can progress to a malignant tumor, *ras* oncogenes have also been detected in several benign tumors which either are self-regressing and/or have a very small probability of progressing to malignant tumors. These tumor types include human skin keratoacanthomas and basal-cell carcinomas (Table 2). Thus, *ras* activation is not sufficient in all cases to promote the continual growth of a tumor, and subsequent accumulation of additional genetic damage is required to make the tumor invasive and metastatic. These examples are not inconsistent with the observation that *ras* oncogenes can induce differentiation in neuronal (*108,109*), endocrine (*110*), lymphoid (*111*), and fibroblast (*112*) cells.

Fourth, the frequency with which *ras* oncogenes are detected in human tumors depends on the differentiation and subtype: for example, *a)* K-*ras* oncogenes are detected in colon villous adenomas and villoglandular polyps but not in papillary adenomas; *b) ras* oncogenes are detected in thyroid follicular and undifferentiated carcinomas but at a much lower frequency in papillary carcinomas; *c)* K-*ras* oncogenes are detected in ovarian mucinous adenocarcinomas but at a much lower frequency in differentiated nonmucinous tumors; and, *d)* K-*ras* oncogenes detected in pulmonary adenocarcinomas may occur predominantly in the subtypes of this tumor that are associated with cigarette smoking (*102*).

Fifth, the correlation between humans and rodents of the role of *ras* gene activation in tumor development is variable (Tables 2 and 3). For human pulmonary adenocarcinomas and pancreatic carcinomas, there are animal models in which K-*ras* oncogenes are involved in the tumor process. Human thyroid follicular and undifferentiated tumors contain all three *ras* oncogenes, and rat thyroid tumors have been induced which contain H- and K-*ras* oncogenes. Rodent and human subcutaneous SCC and basal-cell carcinomas contain activated H-*ras* oncogenes. Human and rabbit skin keratinoacanthomas contain H-*ras* oncogenes. In contrast, *ras* oncogenes are detected at a high frequency in several rodent tumor types but at a very low frequency or not at all in the corresponding human tumors: for example, *a)* H-*ras* in mouse hepatocellular tumors but not in the corresponding human tumors; *b)* H-*ras* in rat mammary adenocarcinomas but not in human mammary tumors; and *c)* K-*ras* in rat lung SSC but not in human lung SSC.

Non-*ras* Oncogenes in Human and Rodent Tumors

Numerous proto-oncogenes other than the *ras* genes have been shown to be activated in human tumors and to a much lesser extent, in rodent tumors. Recently, the classical heterotrimeric G proteins were detected in an oncogenic form in human endocrine tumors (*113–115*). These G proteins are involved in signal transduction pathways. For example, they can mediate the activation of adenylyl cyclase and the production of c-AMP in response to tropic hormones and can also promote the growth of some endocrine cells. Point mutations in the chains of $G_{i\alpha}$ were observed in 3 of 11 tumors of the adrenal cortex and 3 of 10 endocrine tumors of the ovary (*15*). The mutant α gene of $G_{i\alpha}$ is designated *gip*2, a putative oncogene. The α chain of G_s was observed to contain a point mutation in 18 of 42 growth hormone-secreting pituitary tumors (*15*). The mutant α chain of G_s is designated *gsp*. The point mutation in the gene inhibits the GTPase activity of the α protein and thus keeps the G protein in the active GTP complex. Thus, the mechanism of proto-oncogene activation of the α genes is similar to that observed for the *ras* genes.

Induction of aberrant expression of proto-oncogenes by gene amplification or chromosomal translocation has been observed in a variety of tumors (*1,3–5,10–12*). Proto-oncogene amplification is observed as both a low-frequency event in diverse tumor types and as a high-frequency event in specific tumor types. The following examples illustrate the amplification of proto-oncogenes in human tumors.

Neuroblastomas. N-*myc* amplification has been observed in more than 50% of the tumors examined (*116–118*). Amplification of N-*myc* appears to be restricted to the most aggressive tumor cells, and the degree of amplification is inversely related to survival time (*116,118–121*). Clinically, amplification of N-*myc* may be a better prognostic factor than the stage of the tumor (*122*).

Breast Adenocarcinomas. Several proto-oncogenes have been observed to be amplified in this tumor type. The frequencies of increased copy number of c-*myc* range from a low 1–2% (*123,124*) to a much higher 14–48% (*125,126*). Perhaps the most interesting observation has associated c-*myc* amplification with hormone receptor negativity (*125*), high tumor grade (*125*), and older patient age (*126*). Coamplification of *int*-2 and *hst*-1 has been found in 9–23% of breast cancers (*127*). In contrast, amplification of *int*-2 and *hst*-1 is observed most often in steroid-positive tumors and may define a small subset of node-negative breast cancer patients who are at high risk (*127*). The reported amplification rates for HER2/*neu* range from 10 to 46% (*123,124,128–130*). Table 1 in reference *128* summarizes the earlier findings for HER2/*neu* amplification. This proto-oncogene is amplified in both node-negative and node-positive breast cancers (*123,128,129*), even though it tends to be more frequent in patients with larger numbers of positive nodes. The value of HER2/*neu* as a prognostic factor in node-negative patients is controversial (*123,128,129*) and may be limited to a small subset of such patients (*123*). Thus, amplification of various proto-oncogenes appears to play an important role in the development of human breast cancer, although the amplification of a specific oncogene may be limited to a small subset of mammary tumors.

Gastric Adenocarcinomas. Several oncogenes have been found to be amplified in tumors of this type but each

at a relatively low frequency: C-*myc* (4%), *hst*-1 (6%), HER2/*neu* (6%), and K-*ras* (10%) (*131*). Of 50 patients analyzed, at least one of these genes was amplified, and several patients had more than one of the genes amplified. Other studies have shown that HER2/*neu* is amplified in 10% of gastric carcinomas, especially in well-differentiated adenocarcinomas (*132–135*).

Lung Tumors. L-*myc* has been found to be amplified in 5–20% of primary SCLC (*136–139*) but very infrequently in non-SCLC (*136,140*). N-*myc* was amplified in 0–10% of primary SCLC (*136*) but very infrequently in non-SCLC (*136*). N-*myc* and L-*myc* amplification and overexpression are characteristic of SCLC. C-*myc* has been detected as amplified in 0–20% of SCLC (*136–139*) and 10–30% of non-SCLC (*136,139–141*). Thus, amplification of genes of the *myc* family may be involved in the development of some human lung tumors. Overexpression of these genes occurs at a higher rate: N-*myc* overexpression was observed in 46% of SCLC samples in one study (*142*), and c-*myc* was overexpressed in 8 of 12 non-SCLC in another study (*136*). *myc* Gene mRNA overexpression appears to occur more frequently and is observed earlier than gene amplification. Amplification of the *myc* genes is probably not involved in the initiation of human lung tumors but is related to latter stages of tumor progression (*136*).

Esophageal Squamous Carcinomas. Coamplification of *int*-2/*hst*-1 genes was observed in 28% (*143*) and 52% (*144*) of esophageal squamous carcinomas and in 30% (*143*) and 100% (*144*) of lymph node metastases. The survival rate of patients with amplified *int*-2/*hst*-1 genes was significantly lower and the frequency of distant organ metastasis higher than in patients with unamplified *int*-2/*hst*-1 genes (*143*). The protein products of *int*-2/*hst*-1 may act as paracrine growth factors for angiogenesis and thus aid in the metastatic process.

Proto-oncogene amplification is usually associated with progression of neoplasia rather than with initiation of carcinogenesis. Gene amplification is a very rare event in normal cells (*145,146*) and the observed amplification in tumor cells may reflect their genetic instability (*147*). In several recent reports, however, HER2/*neu* was observed to be amplified in early clinical stages; thus, amplification of this gene may also be important in the early stages of some mammary tumorigenesis (*129,130,148*). Whereas gene amplification is almost always accompanied by protein overexpression, some tumors with a single copy of a proto-oncogene may overexpress the protein. The role of gene overexpression, in the absence of gene amplification, in the tumorigenic process is unclear. The overexpression could result from mutations in other genes and thus be regarded as a consequence of the development of the tumor; alternatively, alterations in the regulatory regions of a proto-oncogene, e.g., HER2/*neu* in mammary tumors or *myc* genes in lung tumors, could result in overexpression and thus itself be an important step in the tumorigenesis.

These examples demonstrate that amplification of proto-oncogenes and possibly gene overexpression in the absence of gene amplification are important events in the development of many human tumors. For a specific tumor type, amplification of any one proto-oncogene may occur at a low frequency, but the frequency of tumors in which at least one proto-oncogene is amplified may be relatively high.

Only a few reports have been published of gene amplification in rodent tumors: the c-*myc* gene was amplified in 8 of 12 rat skin tumors induced by ionizing radiation (*149*); the Ha-*ras* gene was amplified as well as mutated in mouse skin tumors induced by 7,12-dimethylbenz[*a*]anthracene (DMBA) (*150*); amplification of H-*ras* and HER2/*neu* was observed in oral cancers induced by DMBA in hamsters (*151*). The paucity of observations of proto-oncogene amplification in rodent tumors may be a reflection of a lack of systematic investigation for amplified proto-oncogenes in malignant tumors.

Genomic rearrangements are commonly encountered in the karyotypes of cancer cells. The significance of these rearrangements has been elucidated in several cases when the altered gene was identified as a proto-oncogene. For example, c-*myc* is joined to various immunoglobulin genes in more than 95% of Burkitt's lymphomas (*152–154*) and murine plasmacytomas (*155–157*). C-*myc* gene rearrangements analogous to those observed in sporadic Burkitt's lymphomas were detected in 12 of 16 cases of AIDS-associated non-Hodgkin's lymphoma (*158*). The translocations probably perturb transcriptional control of c-*myc* and potentiate oncogenic growth of B cells (*159*). During generation of the Philadelphia chromosome, found in more than 90% of chronic myelogenous leukemias (*160*), the tyrosine kinase domain of the c-*abl* proto-oncogene located on 9q is moved to chromosome 22 and joined to an undefined genetic locus, termed *bcr*, to create a hybrid *bcr-abl* protein with oncogenic potential (*161*). Chromosome translocation may indicate the presence and chromosomal location of previously unidentified oncogenes; for example, *bcl*-2 is frequently activated (85–95%) by translocation in follicular lymphomas (*162,163*).

Activated proto-oncogenes other than *ras* have also been detected by DNA transfection methods in both human and rodent tumors (*3,12*). The following examples illustrate the variety of non-*ras* oncogenes detected in the NIH/3T3 focus assay and the nude mouse tumorigenicity assay (*164*): *a)* Two proto-oncogenes that display tyrosine kinase activity, *ret* and *trk*, have been found to be activated by gene rearrangement in human papillary thyroid carcinomas (*51*). The authors originally referred to the rearranged *ret* proto-oncogene as *PTC*; they later determined that *PTC* was derived from rearrangement of an unknown amino-terminal sequence with the tyrosine kinase domain of the *ret* proto-oncogene. The *trk* proto-oncogene is also activated by rearrangement of the tyrosine kinase domain of the gene with an unknown DNA sequence. The *trk* gene was originally discovered in a colon carcinoma as an oncogene derived by rearrangement of a gene with tyrosine kinase activity (*165*). The *ret* oncogene was detected in 9 of 36 papillary thyroid tumors, and the *trk* oncogene was detected in 4 of 16 tumors. Distinct gene rearrangements were observed in each of the tumors that contained one of these oncogenes. *b)* The HER2/*neu* and *hst*-1 proto-oncogenes, which are amplified in some human

tumors, as discussed above, were originally detected by DNA transfection assays. The *neu* oncogene was detected in neuroblastomas induced by *N*-ethyl-*N*-nitrosourea in rats (*166*) and was activated by a point mutation in the transmembrane region of the putative growth factor receptor encoded by *neu*. The *hst*-1 oncogene was isolated from a human gastric carcinoma (*167*). *c)* A transforming gene called *lca* (*168*) was detected in two human hepatocellular carcinomas. *d)* An oncogene designated *axl* was detected in a human myeloid leukemia (*56*); *e)* The c-*raf* proto-oncogene can be activated artifactually during the transfection process; however, in a human pulmonary adenocarcinoma (*45*) and in two mouse hepatocellular carcinomas (*20*), we detected an activated c-*raf* gene in two independent transfection assays. This suggests that the activated c-*raf* may be present in the original tumors. B-*raf* oncogenes were also detected in two mouse hepatocellular carcinomas (*20*). *f)* Several potentially new types of oncogenes that await characterization have been detected in human lung tumors (*45*), mouse lung tumors (*80,90*), rat nasal squamous carcinomas (*169*), mouse lymphomas (*78,170*), and rabbit SCC (*91*).

Recent studies involving the NIH/3T3 focus-forming assay and the nude mouse tumorigenicity assays suggest that some human tumor types contain a high frequency of activated proto-oncogenes. Oncogenes were detected in 62% of papillary thyroid tumors (*51*) and in 12 of 14 (86%) non-SCLC (*45*). The oncogenes detected include both *ras* and non-*ras* genes. Thus, systematic examination of human and rodent tumors by DNA transfection methods may result in the detection of novel and/or previously identified oncogenes at a relatively high frequency.

Summary

The activation and concerted expression of proto-oncogenes are probably involved in the development of most human and rodent tumors. It is very likely that not all of the oncogenes involved in the development of primary tumors have been identified. The accumulation of genetic damage in the forms of activated proto-oncogenes and inactivated tumor-suppressor genes is the driving force in the evolution of a normal cell to a malignant cell. *ras* Oncogenes and mutated *p53* genes have been observed in the same human colon cancer cells (*17*), and it is possible that some human mammary tumors have both a mutated *p53* gene and an amplified and overexpressed HER2/*neu* gene (*129,171*). Earlier studies showed that combinations of oncogenes (i.e., *myc* plus *ras*) could cooperate to transform a cell, whereas each oncogene by itself was incapable of eliciting transformation of a normal cell (*2*). An alternative mechanism, which may operate in the development of many primary tumors, is inactivation of a suppressor gene(s), resulting in inappropriate expression of a proto-oncogene(s); the inappropriately expressed proto-oncogene(s) could then cooperate with an activated proto-oncogene(s) present in the cell to elicit transformation analogous to that induced by *myc* plus *ras*. As additional tumors are examined for both oncogene and suppressor genes, more examples will be detected in which both types of altered gene occur in the same tumor.

The etiology of the genetic alterations observed in most human tumors is unclear at present. The C to T mutations at CpG sites in the *p53* gene detected in human colon tumors may very well occur spontaneously. In contrast, the G to T mutation detected in non-SCLC at various positions on the *p53* gene and in the 12th codon of the K-*ras* gene are probable consequences of cigarette smoking. The mutations in codon 278 of the *p53* gene observed in some aflatoxin B_1-associated tumors could result from chemical exposure. The *ras* mutations in skin tumors at sun-exposed sites are probably induced by ultraviolet radiation. In most cases, however, the genetic alterations observed in tumors are probably generated by both spontaneous events and environmental insults. For sporadic tumors, the initiation step or early genetic alterations may be caused directly or indirectly by exogenous agents, and later events occur spontaneously.

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