# Tobacco Smoke: Chemical Carcinogenesis and Genetic Lesions

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**Lung cancer is the primary cause of cancer-related death in the United States and smoking is the major contributing factor. An amazing array of carcinogenic and precarcinogenic agents has been identified in cigarette smoke. This review addresses the mechanisms by which some of the predominant lung carcinogens mutagenize DNA and explores some of the pivotal genes that are both susceptible to mutagenesis and contribute to the process of tumorigenesis and carcinogenesis. In addition, genes, gene products, and genetic polymorphisms, which curtail the processes leading to carcinogenesis, are discussed. The availability of clinically serviceable methods for analysis of genetic changes that can contribute to lung cancer is imperative to the development of timely, accurate, and cost-effective diagnostic and prognostic tests.**

**A more thorough understanding of the step-wise conversion of precarcinogens to carcinogens, binding of carcinogens to DNA, mutation of DNA, DNA repair, and cellular destruction of carcinogens should contribute to the design of drugs that can modify these processes. In addition, introduction of genes (such as the *p53* tumor supressor gene) into susceptible lung cells may impede carcinogenesis. Early stage clinical trials for the treatment of lung cancer using this and other genes are presently underway.**

## Introduction

Following Dr. Alton Ochsner's landmark observation, first reported in 1939 (1), of a relationship between smoking and lung cancer, a number of investigations have helped to establish, through retrospective and prospective studies, a definitive causal relationship (2-6).

More recently, the molecular and cellular foundations for this correlation have, to some extent, been clarified. Lung cancer is often a multistep, multifactorial disease involving both genetic and environmental components. Clearly, smoking is a major contributing factor; one in ten lifetime smokers develop lung cancer and more than 90% of lung cancers are correlated with smoking (7). Cigarette smoke mediates its carcinogenic effect, at least in part, through specific identified molecules that bind to and mutagenize DNA. Lesions that inactivate or downregulate tumor suppressor genes (the products of which, by definition, suppress tumorigenesis) and lesions that upregulate or inappropriately activate oncogenes (the products of which contribute to tumorigenesis) encourage the development of lung cancer. In addition, individuals may be inherently predisposed or resistant to lung cancer by genetic polymorphisms that influence activation of precarcinogens, catabolism of carcinogens, or DNA repair. As a more complete comprehension of the process unfolds, gene and drug therapies should provide early interventions that prevent dissemination of neoplastic cells and reduce fatalities associated with this devastating disease.

## Developments

### Identification of smoke-related pollutants

Lung cancer is the number one cause of cancer-related death in the U.S. and second only to cardiovascular disease overall. Because lung cancers are extremely angioinvasive and often metastasize from tumors that are too small to be readily detected, the 5-year survival rate after diagnosis is only 12% (8). Early cancer detection is clearly integral to effective treatment.

Approximately 3,800 specific carcinogenic agents (9) have been identified in cigarette smoke including polycyclic aromatic hydrocarbons such as benzopyrene (B(a)P), the activated form of which intercalates and damages DNA (10, 11) (see Figure 1). The active, or carcinogenic, form of BP is (±)-anti-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene or BPDE. BPDE binds to the N2 position of guanine and causes predominantly G:C to T:A transversions and very specific chromatin "hotspots" for DNA mutations (see p134 *Abbreviations, Definitions, and Notes* section for explanations of these and other genetic terms). The transversion may occur by either mispairing of the adducted guanine with adenine or by preferential insertion of adenine opposite the noninstructive modified base (12).

### B(a)P mutagenesis of the *p53* gene

DNA is mutated by cigarette pollutants in a very deliberate fashion that differs, in general, from the mechanism

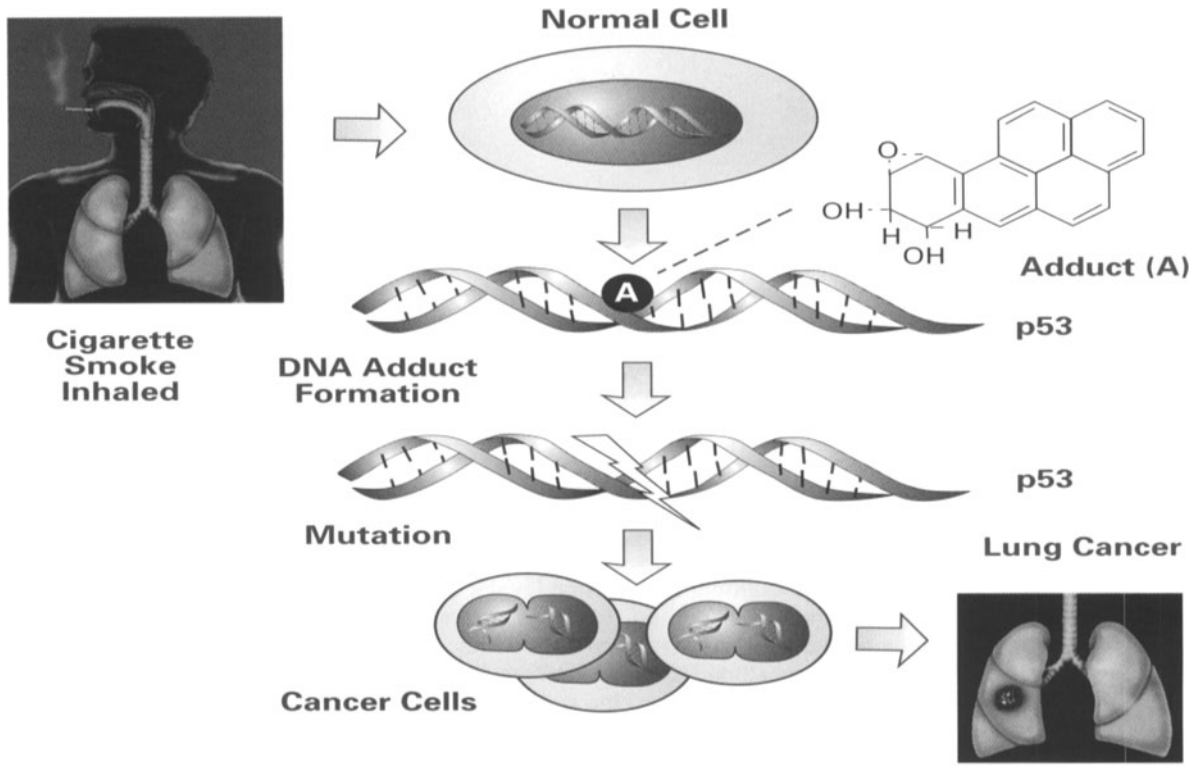


**Figure 1. The carcinogen BPDE (shown as A) binds to guanine residues in DNA and results in mutations. Mutations within tumor suppressor genes and/or oncogenes can contribute to tumorigenesis and lung carcinoma.**

of mutation by endogenous factors. Endogenous factors result primarily in G:C to A:T transitions (13, 14). In many cases these result from methylation followed by deamination of cytosines present in CpG-rich regions of DNA (15). Consequently, the mutation profile of lung cancers in smokers differs from that in nonsmokers. Such is also found to be the case for *p53* mutations in more than 66% of colon cancers; most colon cancers are predicated on endogenous causes (16).

The p53 protein, encoded by a gene located on the short arm of chromosome 17 (17p13.1), is defined as a tumor-suppressor and is believed to mediate its intracellular effect primarily by binding to DNA and modifying transcription from nearby genes. More than 50% of all cancers possess *p53* mutations and 60% of human lung cancers are mutated with respect to *p53* (16). Wild-type p53 protein is believed to behave primarily as a cell-cycle checkpoint protein (see 17 for review). Cellular p53 levels increase through a post-translational mechanism following DNA damage due to endogenous factors, chemicals, or radiation. In turn, it is believed that p53 functions as a transcriptional activator for genes such as *WAF1* (wild-type p53 activated factor 1) and *GADD45* (growth arrested on DNA damage). WAF1 is a cyclin-dependent kinase (CDK) inhibitor that binds to CDK/cyclin complexes and blocks cell cycle progression. Elevation of GADD45, a protein involved in DNA excision repair, can also suspend the cell cycle. Magnified p53 levels, therefore, can stall the cell cycle through elevation of both of these proteins.

Assuming that the cellular DNA repair mechanisms are intact and active, DNA repair may occur, after which p53 levels will diminish and the cell cycle resume. If the DNA repair mechanisms are faulty, or if DNA damage is too extensive, elevated p53 can lead to apoptosis (programmed cell death) (see Figure 2). Rb (the retinoblastoma gene product) can protect cells from p53-induced apoptosis through a yet poorly defined mechanism (18). Consequently, mutation or inactivation of Rb can encourage apoptosis. If p53 is mutated, unable to function as a transcriptional regulator, and unable to mediate apoptosis, cells can continue to divide without DNA repair, chromosomal damage may occur and magnify with each cell division, and neoplasia can ensue (see Figure 2).

Early on it was believed that chemical mutation within the *p53* gene probably occurred at random and that selective growth advantages associated with certain forms of mutated p53 encouraged lung tumor expansion (8). More recently, however, BPDE has been found to bind very specifically to those sites that are hotspots for lung cancer mutation (11). While lung

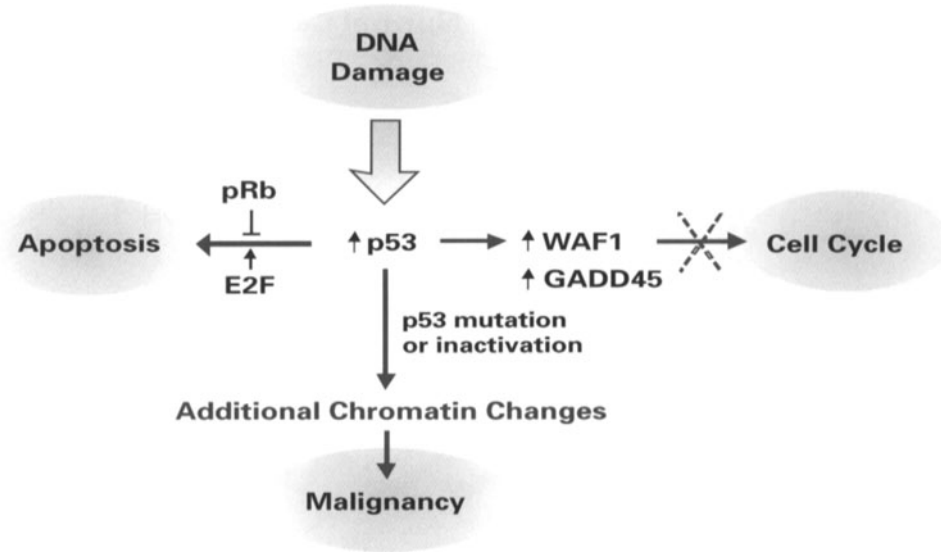


**Figure 2. DNA damage may enhance intracellular p53 protein levels. p53 can stall the cell cycle (through activation of wild-type p53 activated factor [WAF1] and/or growth arrested on DNA damage [GADD45] gene expression) until DNA is repaired, after which the cell cycle may resume. p53 can lead to apoptosis depending on the presence of other intracellular signals. If p53 is absent or mutated, cell cycling can continue in the absence of DNA repair, contributing to the process of tumorigenesis.**

cancer-related mutations occur at more than 100 different bases within the p53 gene, alterations of G residues within codons 157, 248, and 273 together account for more than 90% of lung cancer mutations. Codon 157 is a hotspot specific to lung cancer, while 248 and 273 are frequently mutated in a number of other tumor types. All of these sites lie within the DNA-binding domain. The basis for the specificity of BPDE binding to these specific G residues (as opposed to other G residues) is unclear. However, three facts are clear: 1) the G residues are within CpG couples and the associated cytosines are methylated, 2) mutations occur largely on the non-transcribed strand [repair is known to be deficient on this strand (19, 20)], and 3) binding may be unrelated to chromatin structure since the same pattern has been observed in HeLa cells, bronchial cells, and normal human fibroblasts (11).

The use of p53 as a prognostic indicator for lung cancer is still questionable. A tenuous link has been established between abnormal p53 expression and resistance of non-small cell lung carcinomas (NSCLCs) to cisplatin treatment (21). [NSCLCs account for 75-80% of lung tumors and include adenocarcinomas (AC), squamous cell carcinomas (SC), and large cell carcinomas (22)]. This observation may reflect inactivation of p53-dependent apoptotic mechanisms.

## K-*Ras* mutations and lung cancer

RAS proteins are membrane-bound GTPases. They are signal transducers that catalyze the conversion of GTP to GDP in conjunction with specific regulatory proteins (23, 24), and mediate communication between plasma membrane effectors and genes. While wild-type RAS proteins are involved in cell growth and differentiation, mutations and inappropriate expression of the corresponding genes are highly oncogenic. While *Ras* oncogenes occur in a wide variety of human tumors with an overall incidence of 10-15% (25), the incidence in some tissues, e.g. pancreatic carcinomas, is as high as 95% (26). K-*ras*, the protooncogene of Kirsten murine sarcoma virus (Ki-MuSV) is encoded on the short arm of chromosome 12 (12p12.1).

The most frequent genetic mutations in lung cancer occur in the *FHIT* gene (discussed in the following section), the *p53* tumor suppressor gene, and the K-*ras* protooncogene (27). K-*ras* mutations are found in about 50% of lung cancers, are approximately equally distributed between AC and SC, and are most prevalent in exon 1 (28). As is described for the *p53* gene, a prevalence of G to A transitions occurs in nonsmokers. Transversions are particularly prevalent in SC and rare in AC. It is hypothesized that the airway epithelial cell precursors for SC are more susceptible to tobacco carcinogens. On the other hand, since most lung cancers of non-smokers are adenocarcinomas (29), the airway precursors for these cancers may be more susceptible to mutation by endogenous genetic factors. K-*ras* mutations have been correlated with poor survival and may be of prognostic value (30). In particular, mutations of Gly (codon 12) to Asp or Val have been correlated with very poor survival. Codon 12 is the site of the majority of K-*ras* point mutations, permitting simplified detection assays in most instances.

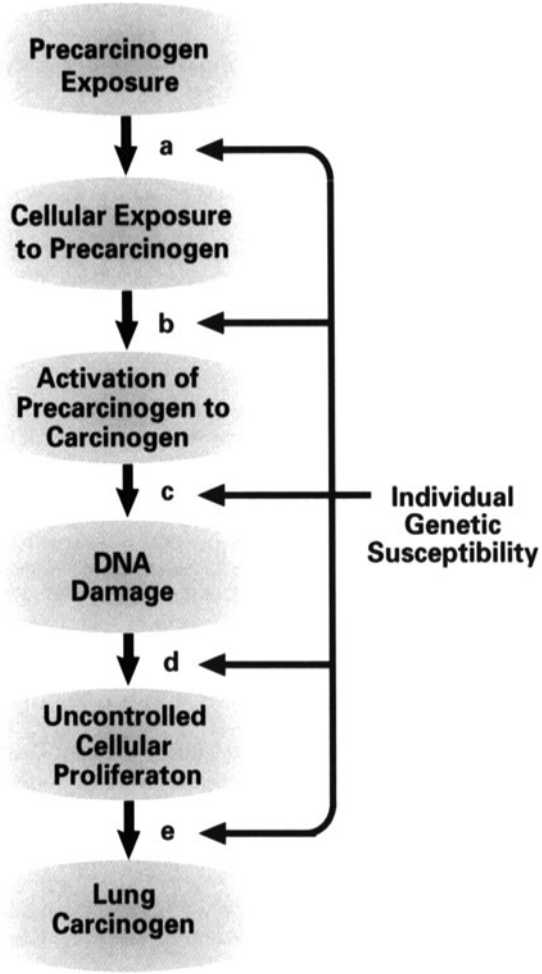


**Figure 3. Steps leading from precarcinogen exposure to lung cancer. Specific polymorphic proteins, active at each step a-e, may enhance cancer susceptibility while others may heighten resistance.**

## *FHIT* mutations and lung cancer

The *FHIT* gene (fragile histidine triad), a tumor suppressor gene located on the short arm of chromosome 3 (3p14.2), is affected by somatic deletions (loss of heterozygosity, LOH) in lung cancer. It represents the most frequent alteration in NSCLCs (>70%) (31). In one study, p53 mutations and LOH at the *FHIT* locus were found to be completely concordant in nonsmokers and unrelated in lung cancers of smokers (32). In a second study, of 474 tumors analyzed, the percent defective with respect to *FHIT* expression was greatest in SC (87%) compared with AC (57%). Large cell carcinomas demonstrated a comparatively intermediate level of *FHIT* defects (69%). In both of these cited studies, the overall frequency of *FHIT* LOH was found to be higher in tumors of heavy smokers.

## Genetic predisposition to lung cancer

As mentioned earlier, lung cancer is often a multifactorial disease possessing both genetic and environmental components. Individual susceptibility is a function of cellular susceptibility, metabolic activation of the precarcinogen, catabolic destruction of the carcinogen, DNA susceptibility, and DNA repair (see 8 for review, Figure 3). Various genetic polymorphisms may affect the efficiencies of any or all of these processes. For instance, CYP1A1, a member of the cytochrome P450 family of aryl hydrocarbon hydroxylases (AHH) can activate B(a)P to BPDE. [CYP1A1 is found in the nonciliated bronchiolar epithelial (Clara) cells, alveolar type 2 cells, and alveolar macrophages (33). Clara and Type 2 cells are postulated as the progenitors for NSCLC (8).] CYP1A1 is present at low levels in the lung but induced by cigarette smoke. Higher AHH levels have correlated with higher DNA adduct levels. Furthermore, higher AHH inducibility has been linked to increased susceptibility to carcinogen-induced lung tumors in experimental animal models (34, 35). Several polymorphisms have been revealed, some of which may serve as markers for lung cancer predisposition.

In addition, metabolism of carcinogens such as BPDE to less reactive forms, involves enzymes including glutathione S-transferase (GST) and NADPH-quinone oxidoreductase (NQO). GSTs conjugate electrophilic xenobiotics with glutathiones (36, 37); specifically BPDE is conjugated with glutathione by GST. Glutathione conjugates are readily excreted. NQOs catalyze the reduction of quinones (very reactive molecules abundant in cigarette smoke) to hydroquinones (significantly less reactive) (38-40). Polymorphisms reported in both of these families of enzymes (8) may affect catabolism of tobacco carcinogens and, thus, lung cancer predisposition.

## Summary

Alterations at the *FHIT*, *p53* and *Ras* loci, as detectable in cells from sputum or bronchial lavages, could be used for early detection in screening programs. Elevated levels of RAS have been detected in serum of cancer patients possessing *Ras* tumor mutations, suggesting the basis for cancer screening tests (41, 42). Animal experimental data suggest that *Ras* mutations are very early events in lung carcinogenesis, occurring within days to weeks after exposure to tobacco smoke (43, 44). In addition, carcinogen/DNA adducts are the net effect of mutagen exposure, absorption, activation, deactivation, and catabolism and therefore represent potential markers for early detection (8). Reliable detection of DNA/carcinogen adducts, particularly in peripheral blood leukocytes, could be instrumental in early detection and intervention programs.

## Abbreviations, Definitions, and Notes

G = guanine, a purine nucleotide
A = adenine, a purine nucleotide
C = cytosine, a pyrimidine nucleotide
T = thymine, a pyrimidine nucleotide

Under typical Watson-Crick base-pairing conditions, purines pair with pyrimidines through hydrogen-bonds to generate:

G:C to T:A transversion. Transversion refers to the substitution of a purine for a pyrimidine or a pyrimidine for a purine (nonconserved). Transition refers to the substitution of a purine for a purine or a pyrimidine for a pyrimidine (conserved substitution).

Hotspot: a preferentially mutated nucleotide base.

CpG: cytosine-phosphate-guanine. Cytosines within CpG-rich regions of DNA are often preferentially methylated, a process known to modulate gene transcription.

Note that the names of genes and gene products are very similar or identical. However, the gene is conventionally italicized to differentiate it from the corresponding protein.

LOH: Loss of heterozygosity. Chromosomal deletion results in the loss of one allele from a heterozygote, leading to the expression of only the one remaining allele. LOH is detectable by molecular genetic analyses.

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